Ergotoxine and some other constituents of ergot : by George Barger and H.H. Dale.

Contributors

Barger, George, 1878-1939.
Dale, Henry H. 1875-1968.
Wellcome Physiological Research Laboratories.

Publication/Creation

London : Wellcome Physiological Research Laboratories, [1907?]

Persistent URL

https://wellcomecollection.org/works/nxpbhqa3

License and attribution

This work has been identified as being free of known restrictions under copyright law, including all related and neighbouring rights and is being made available under the Creative Commons, Public Domain Mark.

You can copy, modify, distribute and perform the work, even for commercial purposes, without asking permission.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

No. 10

LONDON SCHOOL OF TROPICAL MEDICINE
NOT TO BE TAKEN AWAY.

10

ERGOTOXINE
AND SOME OTHER CONSTITUENTS OF ERGOT

BY

GEORGE BARGER, M.A. (CANTAB.), D.SC. (LOND.)

AND

H. H. DALE, M.A., B.C. (CANTAB.)

(From the Bio-Chemical Journal, Vol. II., p. 240, — 1907)

LONDON SCHOOL OF TROPICAL MEDICINE LIBRARY

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
BROCKWELL HALL
HERNE HILL
LONDON, S.E.

[*Reprinted from the* BIO-CHEMICAL JOURNAL, VOL. II, NOS. 5 AND 6]

ERGOTOXINE AND SOME OTHER CONSTITUENTS OF ERGOT

BY G. BARGER, M.A. (*Cantab.*), D.Sc. (*Lond.*), AND H. H. DALE, M.A., B.C. (*Cantab.*).

From the Wellcome Physiological Research Laboratories, Herne Hill, London, S.E.

(*Received April 3rd*, 1907)

CONTENTS

In papers already published by us (partly in conjunction with F. H. Carr) we have described certain physiological effects produced by ergot preparations and the isolation and chemical properties of the alkaloid ergotoxine, to which these effects are due. In the present paper[1] we propose to describe more completely the general physiological action of this alkaloid, and to discuss, in the light of our earlier experiments, its occurrence in the numerous substances which have from time to time been described as active principles of ergot.

1. For the chemical experiments G. Barger is responsible, for the physiological H. H. Dale.

Historical

Though ergot occupied the attention of a number of chemists in the eighteenth century and the earlier part of the nineteenth—Vauquelin (1), Pettenkofer (2)—the first careful investigation was made by Wiggers (3) in 1831, at a time when the long controversy as to the therapeutic utility of the drug was drawing to a close. Wiggers found 35 per cent. of oil, a crystalline wax-like substance, which he termed cerin, and which is probably identical with the 'ergosterine' of Tanret, a new sugar, later found to be identical with trehalose, and phosphates. He also proved that, contrary to the statements of some of his predecessors, starch and hydrocyanic acid are absent, and described a resin, soluble in alcohol but insoluble in ether and in water, which he termed 'ergotin.' From feeding experiments on cocks he concluded that the toxic properties of ergot are wholly due to this resin. The therapeutic activity he regarded as due to a water-soluble substance, on account of the good effects of liquid (aqueous) extracts when used in medicine—an opinion which still finds many supporters.

Considering the state of chemical knowledge at the time, Wiggers' analysis was so complete that it satisfied chemists for a generation. With the exception of Bonjean (4), who in 1842 described a method for preparing an aqueous extract, which he likewise called ergotin, and which has been adopted in some form or other in most pharmacopoeias, no substantial advance was made till Wenzell (5), in 1864, obtained two fixed alkaloids from ergot, 'ergotine' and 'ecboline.' This discovery led to researches by Manassewitz (6), Herrmann (7), and Ganser (8), who for the most part confirmed Wenzell's results. The whole question, however, was still in a state of great confusion, for in 1874 Buchheim (10) attributed the activity of ergot not to any specific substance, but to the 'putrid and septic substances' as a whole. In striking contrast to this work is that of Tanret (12), who, a year later, made the first great step by discovering a crystalline alkaloid which he prepared in a state of undoubted purity, and named ergotinine (to distinguish it from the resinous 'ergotines' of his predecessors). Tanret's alkaloid has been found by all subsequent

investigators, but has not always been properly identified. Dragendorff and Podwyssotski (14) in 1877 described as 'picrosclerotine' an alkaloid which was undoubtedly identical with ergotinine, as may be inferred from the work of Dragendorff's pupil, Blumberg (15). Other synonyms for the crystalline ergot alkaloid are: sclerocrystalline, (Podwyssotski (17), 1883) and secaline (Jacobj (37), 1897). In 1894 it was erroneously described as identical with Kobert's cornutine by Keller (32), who afterwards, however, abandoned this view.

Tanret regarded ergotinine as the therapeutically active principle, a belief which led Yvon (13) in 1877 to prepare an 'ergotin,' the first of a new type of extracts rich in alkaloids. Tanret's view as to the activity of ergotinine did not, however, meet with universal acceptance, and the search for an alkaloid as the active principle was followed by a period when most investigators looked for a water-soluble acid as the active substance. The starting point of this series of investigations is the '*ergotinum dialysatum*' of Wernich (9), a water-soluble extract prepared in 1874. In 1875 Zweifel (11) described a preparation to which the name 'ergotinic acid' was later applied. Dragendorff and Podwyssotski (14) in 1877 called a similar water-soluble principle 'sclerotinic acid,' which was further referred to by Podwyssotski (17) in 1883, and by Denzel (19) in 1884.

In 1884 Kobert (20) published an elaborate investigation of ergot. In the main his results may be said to be a combination of the 'alkaloidal' and the 'acidic' view, for, of the three active substances described by him, two are acids and one is an alkaloid. For one of the acids, which is soluble in water, he retained the old name ergotinic acid. According to Kobert, it is a nitrogenous, glucosidic substance, and is the chief constituent of the sclerotinic acid of Dragendorff and Podwyssotski. It lowers the blood pressure and paralyses the central nervous system, but does not produce gangrene; it is without action on the uterus, and does not cause vaso-constriction. Chemically, ergotinic acid was regarded by Voswinkel (29) as a carbohydrate (mannane), but Kobert's view was upheld by his pupil Kruskal (31). Quite recently it has been described by Kraft (46) as a mixture containing, among other things, mannite and a new

crystalline acid (secale-amino-sulphonic acid). Since, however, Kobert's statement that it is therapeutically useless has not been disputed, it need not concern us further.

The other substances described by Kobert are both insoluble in water, but soluble in alcohol. They are 'sphacelinic acid' and the alkaloid 'cornutine.' According to Kobert both substances produce contractions of the uterus and act on the vaso-motor centre, causing rise of blood-pressure. In these respects relatively large doses of the acid correspond to small doses of the alkaloid. The chief points of difference are that sphacelinic acid produces gangrene, and cornutine does not, and that cornutine, in small doses, has a convulsant effect superficially similar to that of strychnine. Kobert does not claim to have isolated his substances in even an approximate state of chemical purity. The one substance claiming to be an active principle which had at that time been obtained chemically pure, namely, Tanret's crystalline ergotinine, he declared to be inactive.

Kobert's theory of the existence of two principles, sphacelinic acid and cornutine, was upheld by Bombelon (26), though this author's preparations were not examined physiologically. Kobert's results were further confirmed by his pupils; in the case of sphacelinic acid by Grünfeld (30), and in that of cornutine by Lewitsky (24). Evidence both in favour of and against the therapeutic use of cornutine was adduced by various clinical observers. (See, for instance, Erhard (22), Graefe (23), Thomson (27).) In 1889 Kobert (28) contributed to the *Real-Encyklopädie der Pharmacie* his article on ergot, in which he gave a modified method for the preparation of cornutine, and strongly recommended this alkaloid for obstetrical purposes, whereas in 1884 he inclined to favour the use of sphacelinic acid.

Keller (32) in 1894 adopted this later view of Kobert, and, further assuming that ergotinine and cornutine are the same and the only alkaloid in ergot, he based a method of assay on the determination of the total alkaloid. For this supposed one alkaloid he preferred the name cornutine 'on practical grounds.'

Later, in 1896, Keller (35) regarded cornutine as a partially decomposed ergotinine, and advanced arguments against Kobert's

view that ergotinine is inactive. These arguments are based on the activity of commercial (impure) ergotinine specimens, and prove nothing as to the activity of the chemically pure base—a point with which we shall deal in a later section of this paper.

The substance described as 'cornutin' by Keller was examined physiologically in 1902 by Santesson (39), who used a specimen obtained from Keller himself, and others prepared according to the latter's directions. In frogs, rabbits, and fowls he obtained with this preparation, in considerable doses, only a partial and feeble reproduction of some of the effects attributed by Kobert to sphacelinic acid and cornutine. A significant rise of blood-pressure was obtained only in the fowl, and the effect on pregnant rodents was not of a constant or definite nature. He concludes that this substance is not the important active principle.

Up till 1906 two further attempts to isolate the active principle were made by Jacobj (37) and by Meulenhoff (38). Both these investigators adopted as their chief criterion of activity the reaction of the cock's comb, which Kobert only obtained with sphacelinic acid. According to Jacobj the active principle is a non-nitrogenous resin, with feebly acid properties, for which he adopted the name 'sphacelotoxin,' and which he described as combined in ergot with two inert substances—(*a*) with 'ergochrysin,' to form the compound 'chrysotoxin'; (*b*) with the crystalline alkaloid 'secaline' to form the compound 'secalintoxin.' Sphacelotoxin Jacobj regarded as sphacelinic acid in a pure form. Both chrysotoxin and secalintoxin caused uterine contractions and gangrene of the cock's comb. Meulenhoff likewise concluded that the activity of ergot is due to an acid resin (Kobert's sphacelinic acid). With regard to cornutine, Meulenhoff confirmed Tanret's view that it is a decomposition product of ergotinine formed by the acid used in its extraction, and does not occur in ergot as such.

It is evident from the above account that when, a few years ago, we began to work on ergot the more recent invetigators had held that the activity of ergot, or, at any rate, the production of gangrene, was determined by an acidic principle. Our own

experiments have, however, led us to believe that all these acidic preparations owed their activity to a powerfully active amorphous alkaloid, of which crystalline salts were isolated by F. H. Carr and one of us, and to which the name ergotoxine was given (45). Soon afterwards Kraft (46) described the same alkaloid under the name hydroergotinine, regarding it as the hydrate of Tanret's ergotinine. Recently ergotoxine and some of its salts have been described in detail by Barger and Carr (51), who, from their analyses, assign to ergotoxine the formula $C_{35}H_{41}O_6N_5$, and to ergotinine the formula $C_{35}H_{39}O_5N_5$, thus establishing Kraft's view as to the relationship of the two alkaloids. Meanwhile a substance of an entirely different kind, neither acidic nor alkaloidal, was described by Vahlen (44) as the essential therapeutic principle of ergot. With the nature of this substance, to which he gave the name 'clavin,' we shall deal in a later section of the paper.

Ergotoxine

Chemical

The chemical description of the alkaloid ergotoxine, which has already been given elsewhere by Barger and Carr (51), may be summarised as follows :—Ergotoxine is a white amorphous powder having the composition $C_{35}H_{41}O_6N_5$, and melting, with decomposition, at 162° to 164°. It is freely soluble in most organic solvents, but only slightly so in ether, and is insoluble in light petroleum. It is soluble in dilute caustic soda, and is a feeble monacid base. Ergotoxine forms crystalline salts, one of the most characteristic of which is the phosphate $C_{35}H_{41}O_6N_5$, H_3PO_4, H_2O, forming minute needles, melting at 186° to 187°.

Barger and Carr have amended Tanret's original formula for crystalline ergotinine to $C_{35}H_{39}O_5N_5$. (Compare with this Tanret's recent formula, $C_{35}H_{40}O_5N_5$ (49).) Hence it will be seen that the crystalline alkaloid is the anhydride of the amorphous, as first suggested by Kraft. Either alkaloid can be readily converted into the other. Both give the colour reaction described by Tanret and by Keller as

characteristic of ergotinine. The chief differences between the two alkaloids are that ergotinine crystallises very readily, whereas ergotoxine has so far resisted all attempts at crystallisation, and that ergotoxine is very soluble in cold alcohol while ergotinine is but slightly soluble. So far only amorphous ergotinine salts have been prepared, whereas nearly all the ergotoxine salts hitherto examined have been obtained crystalline. The salts of both alkaloids form colloidal solutions in water, and are precipitated by electrolytes, so that they are little soluble in the presence of the stronger mineral acids.

Physiological

One of us recently (43) described certain physiological effects—best observed in a cat with the brain destroyed and artificial respiration—which were characteristically produced by a large number of ergot preparations. An analysis of these effects showed that they could be divided into—

(1) A primary stimulation of plain muscular tissues, especially the arteries, the uterus, and the sphincter of the pupil.

(2) A secondary specific paralysis of the motor elements in the so-called ' myoneural junctions ' associated with innervation by the true sympathetic system and stimulated by the suprarenal active principle; the inhibitor elements of the same retaining their normal function, as do also the autonomic nerves of cranial and sacral root origin.

The vaso-motor effects may be taken as a typical and easily observed example of this double action. When a powerful dose of one of these ergot preparations (chrysotoxin, commercial ergotinine, etc.) is injected intravenously into a pithed cat the first result is a marked and very prolonged rise of blood-pressure. If, while this rise persists, the sympathetic nerve supply to the arteries is excited at any level, as by faradising the spinal cord or the splanchnic nerves, or injecting intravenously nicotine or a suprarenal preparation, the effect is a very marked *fall* of blood-pressure in place of the customary rise. This particular instance of the action is easy to observe, and is capable

of quantitative application, and we shall frequently refer to the dose of a given preparation causing ' vaso-motor reversal,' meaning thereby the quantity just sufficing to replace the normal pressor effect of a given dose (0·1 mgm.) of the suprarenal active principle by a depressor effect. The accuracy of the measurement is admittedly not great, but it has, at least, a fairly definite end-point, and we have found it preferable to observation of such uncertain effects as those on the cock's comb. At the outset it was necessary to recognise the possibility that more than one active principle might be concerned in the various actions, and for a long time we were engaged in the search for a principle of which we could only postulate that it caused the vaso-motor reversal. Only later were we able to transfer conclusions based on this reaction to the other physiological effects, which the principle, when isolated, was found to produce. In the former communication it was suggested as probable that the primary stimulant action on plain muscle was due to a different principle from that responsible for the secondary sympathetic motor paralysis, although the two were found in close association. This suggestion has recently been supported by Cushny (50), who observed, as we did, that certain pharmacopoeial preparations, such as the liquid extract, produced stimulant effects on plain muscle, resembling, superficially at least, those which we had described, but followed by a disproportionately weak sympathetic motor paralysis. Like ourselves, he regarded this as indicating that the principle responsible for the paralytic effects probably had none of the stimulant properties, and that, where the two sets of effects were observed, two principles were at work. The isolation of ergotoxine, in the form of pure crystalline salts, at once showed, however, that this conclusion was wrong. What, if any, is the relation to ergotoxine of the substance which gives to the liquid extract what specific activity it possesses is at present quite uncertain, and will probably remain so until the substance can be obtained free from other physiologically active principles, such as choline. Possibly the further experiments on ergotoxine, with which we are now engaged, will throw light on the question. However that may be, it is certain that pure salts of ergotoxine produce, in very small dose,

all the effects described in the former paper (43) as characteristic of chrysotoxin, etc.

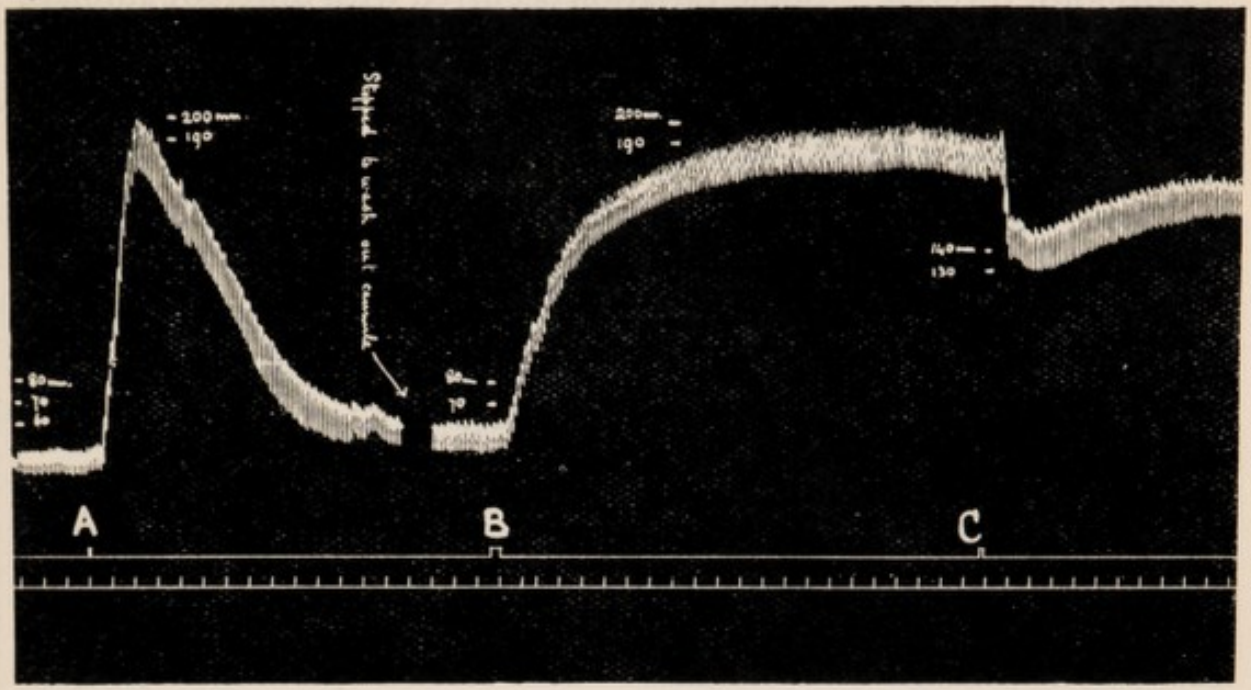


FIG. 1

Pithed Cat, 2 kilos. Artificial respiration. Carotid blood-pressure.
At A—Intravenous injection of 0·5 mgm. of the suprarenal principle.
At B—Intravenous injection of 1 mgm. of ergotoxine phosphate.
At C—Intravenous injection of 0·05 mgm. of the suprarenal principle.
Time marker in this and all the other tracings showed ten seconds intervals.

Reference to that paper will show that, at the time of its publication, we had already found that certain alkaloidal preparations produced the effects in smaller dosage than those of an acidic, resinous nature. The only step needed was the isolation of the alkaloid, in chemically pure form, which was made possible by the discovery that it gave well-crystalline salts. Without repeating the details of the various manifestations of the physiological action already described, it will be sufficient to indicate the relative activity of the pure alkaloid as compared with the impure preparations previously used. As with these impure preparations it was found that, owing probably to the depressant action of the alkaloid on the medullary centres, the stimulant effects were observed in most characteristic form in an

animal with destroyed brain, and under artificial respiration. In a cat under these conditions, 0·5 mgm. of a pure ergotoxine salt per kilo caused the characteristic marked rise of blood-pressure, succeeded by 'vaso-motor reversal.' Some variation occurred with different animals, but the dose necessary to reverse the vaso-motor effect of 0·1 mgm. of the suprarenal principle in the cat did not, in any case, differ widely from this. Rather more—about 1 mgm. per kilo.—was needed to reverse the motor effect of suprarenal or other sympathetic stimulation on the uterus of the cat in early pregnancy: in a case of later pregnancy the dose needed was larger. As with the impure preparations, the sympathetic effects on the heart and the dilator of the iris were comparatively resistant to the paralysis. No quantitative determinations were made with other animals, but, in addition to the species previously examined with the impure preparations (cat, dog, rabbit, ferret, monkey and fowl), observations were made on the pig and the goat.

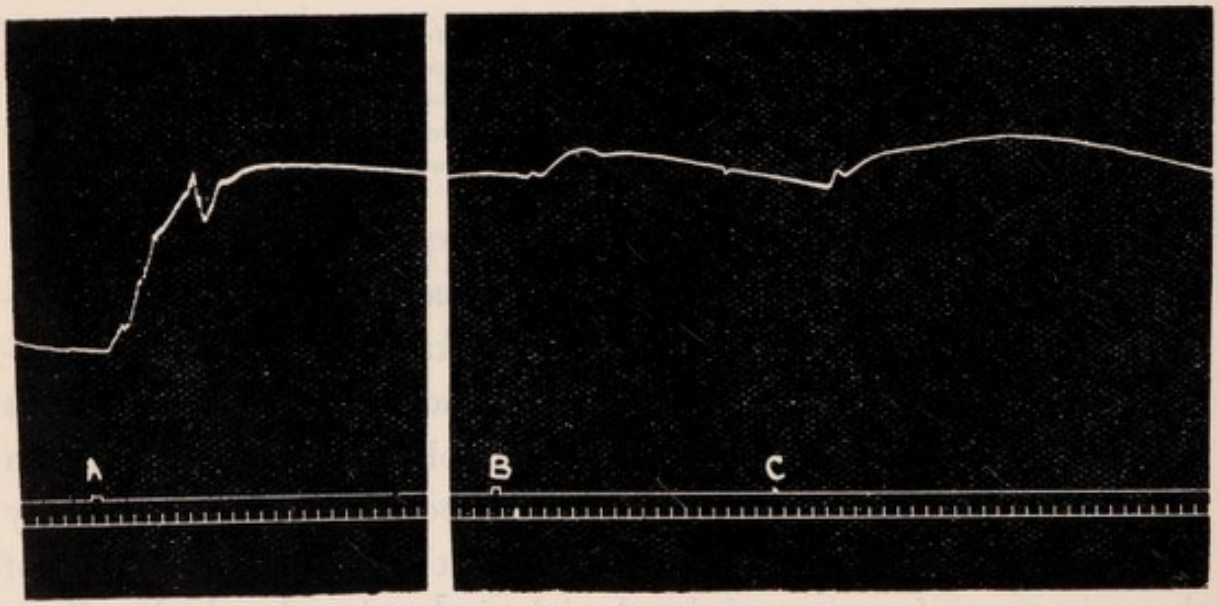


FIG. 2

Cock, 2 kilos. Brain pithed. Artificial respiration. Carotid blood-pressure. Injections intravenous.
At A—5 mgms. of ergotoxine in dilute caustic soda.
At B—5 mgms. of ergotoxine in dilute caustic soda.
At C—0·05 mgms. of the suprarenal active principle.
Note that even 10 mgms. have not obliterated or reversed the suprarenal effect.

In a small pig of 9·5 kilos., with brain destroyed and artificial respiration, intravenous injection of 4 mgms. of ergotoxine phosphate, dissolved in water, caused a rise of blood-pressure from 90 mm. to 140 mm. of mercury ; a further dose of 4 mgms. produced no further pressor effect, and a subsequent injection of 0·1 mgm. of the suprarenal principle caused no longer any rise of blood-pressure, though, on the other hand, no perceptible depressor effect was produced. It appears probable, then, that the vaso-constrictor mechanism in the pig is nearly as sensitive as that of the cat to ergotoxine-paralysis, but that the vaso-dilator element in the sympathetic is insignificant or absent. The pig's bladder gave after, as before the administration of ergotoxine, an inhibitor response to suprarenal and to stimulation of the hypogastric nerves. In the goat the experiment was made under anaesthetic (A.C.E. mixture), with the brain intact. A less marked rise of blood-pressure was produced by injecting ergotoxine phosphate, but the abolition of pressor effect of the suprarenal principle followed, as in the pig, and, moreover, its normal motor effect on the goat's bladder was replaced, after ergotoxine, by distinct inhibition. As pointed out in the former paper, the vaso-motor supply of the cock is particularly resistant to the ergotoxine paralysis. (See Fig. 2.)

Besides the question of the identity of the principles producing the 'stimulant' and 'paralytic' effects respectively, another point was left in doubt in the former paper, namely, the point of action of the stimulus producing the arterial constriction. It was stated there that the effect was produced somewhere peripheral to the spinal cord, and that the slight effect of chrysotoxin, etc., after large doses of nicotine suggested an action on the ganglion cells of the sympathetic system, while, on the other hand, the persistence of the pupillary constriction after atropine indicated, in the case of the *sphincter iridis*, a quite peripheral action. A repetition of the nicotine experiment with pure ergotoxine salts has shown that the pressor effect is quite well produced in the cat after doses of nicotine (30 mgms.) sufficient to abolish the ordinary effects of stimulating sympathetic nerves (such as the pupillo-dilator action of the cervical sympathetic), provided only that the action of the heart has not been too greatly enfeebled by the nicotine injections. (See Fig. 3.)

This indication that the stimulant action on the arteries was also peripheral in origin was confirmed by a plethysmographic experiment. In a cat, with pithed brain and artificial respiration, the right stellate ganglion was excised extrapleurally by Anderson's method.[1] The cat was then turned over, the right fore limb enclosed in a glass plethysmograph, connected with a small Hürthle piston-recorder,

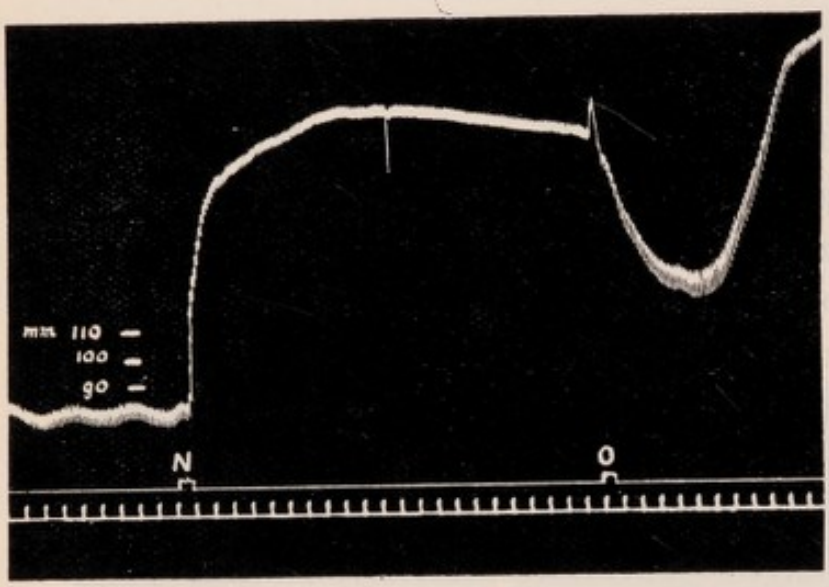


FIG. 3

Cat, 3 kilos. Pithed. Artificial respiration. Thirty mgms. of nicotine had been given. Injections into external jugular.
At N—1 mgm. of ergotoxine phosphate in water.
At O—0·1 mgm. of the suprarenal active principle.

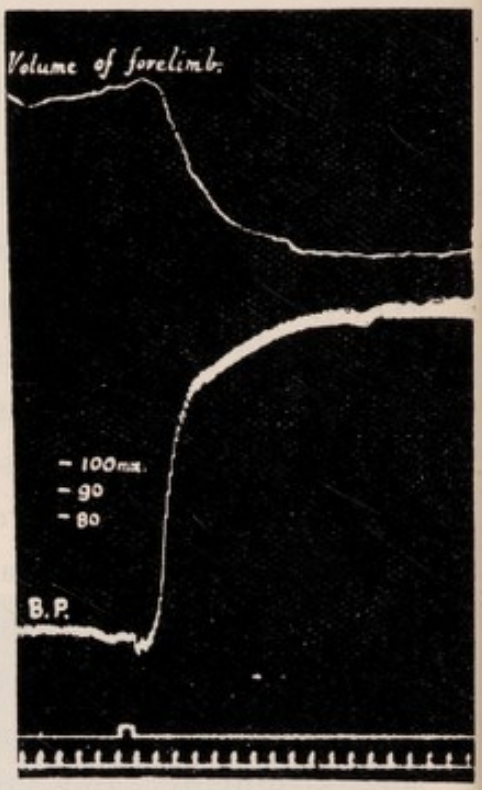


FIG. 4

Cat, 3 kilos. Pithed. Curare. Artificial respiration. Right stellate ganglion excised. Right fore limb in plethysmograph. Carotid blood-pressure.
At point signalled, 2 mgms. of ergotoxine phosphate, in water, was injected into the jugular vein.

and the blood-pressure recorded from the carotid artery. Two mgms. of ergotoxine phosphate, dissolved in water, were then injected into the external jugular vein. The result is shown in Fig. 4. It will be

1. *Journ. of Physiol.*, XXXI, p. 21 (Proc. Phys. Soc.), 1904.

seen that the rise of general blood-pressure is accompanied by a simultaneous decrease in the volume of the fore limb, which can only have been caused by active constriction of the arteries. As the stellate ganglion had been completely extirpated, the action of the ergotoxine in producing the arterial constriction must have been peripheral on the muscular walls of the arteries themselves. This is in accordance with the result obtained by Jacobj (37) in a perfusion experiment with his preparations. On the other hand, Kobert (25) observed no indication of any effect on the vessels of an excised kidney perfused with a solution of ergotinine in blood, and Dixon (42) found some dilation instead of constriction of peripheral vessels perfused with a liquid extract of ergot diluted with Ringer's solution. It will be clear from what follows that the significance of Kobert's experiment depends largely on the purity of the ergotinine used, and it has already been pointed out that experience obtained with a liquid extract cannot properly be applied to ergotoxine.

There remains the question as to which structure in the arterial wall is the seat of the action. It was pointed out in the former paper that a large dose of an active preparation reverses or obliterates the effect not only of a subsequent suprarenal or nicotine injection, but of a further injection of the ergot preparation itself, whereas barium chloride and pituitary extract still produce their normal actions. This suggests that the stimulant action of ergotoxine is on those elements, the sympathetic motor myoneural junctions, which it subsequently paralyses. Such a conclusion would, of course, be expected on general grounds, and it is supported by the consideration that the stimulant effects are most conspicuous in the case of plain muscular organs which are known to receive motor fibres from the true sympathetic system, viz., the arteries and the uterus. The absence of the pressor effect of ergotoxine after large doses of apocodeine (250 mgms. for a cat) points in the same direction, though the significance of the observation is weakened by the fact that we have never, with the specimens of apocodeine at our disposal, succeeded in obliterating the suprarenal rise of blood-pressure without, at the same time, markedly weakening the action of the heart. On the

other hand, ergotoxine very distinctly stimulates plain-muscular organs in which there is no reason to suspect motor innervation from the true sympathetic. Thus it causes a very marked constriction of the cat's pupil, and a variable contraction of the urinary bladder in the same animal. In the case of the sphincter of the iris the motor nerve-supply from the cranial nerve can be excluded completely by atropine, and there is no reasonable escape from the conclusion that the ergotoxine here acts directly on the muscle-fibres, and the same very probably holds good for the less marked effects on the urinary bladder and the stomach and intestines. But, whatever may be the exact point of action of ergotoxine, there is no good reason for regarding any of its effects on plain-muscular organs as other than peripheral in origin. The effect on the heart itself appears to be a slight one. In a few experiments made with the isolated hearts of rabbits and cats, perfused with Locke-Ringer solution, a change to the same fluid containing 1 in 50,000 ergotoxine hydrochloride caused, in some cases, a small increase in the force of the beat; in some cases no obvious effect. In the anaesthetised animal, with medullary centres and vagi intact, ergotoxine causes an obvious vagus-inhibition of the heart, similar to that which Kobert observed with cornutine. Even with the medulla destroyed, or the vagi cut, the sudden and prolonged rise of blood-pressure, unaccompanied by any marked action on the accelerator mechanism, often produces a marked irregularity of the beat, unless large doses of atropine have been given.

Apart from these effects on decerebrate or anaesthetised animals, the general toxic effects have been studied by injection into frogs, fowls, rabbits, and cats. The action on frogs is of importance in considering the relation of ergotoxine to other ergot alkaloids. According to Kobert, cornutine has a very characteristic action on frogs, producing in doses of as little as $\frac{1}{8\frac{1}{2}}$ mgm. strychnine-like spasms, succeeded by a veratrine-like paralysis. The effects of ergotoxine are very slight as compared with those attributed to cornutine. One to two milligrammes of ergotoxine phosphate dissolved in distilled water, or of the free base dissolved in dilute sodium hydrate, caused an initial trace of increased excitability, succeeded, in the course of a

few minutes, by a diminution in the power of flexing the hind limbs. The following record shows the sequence of events :—

Frog, 29 grammes.

12.40 p.m. 1 mgm. of pure ergotoxine phosphate, dissolved in 1 c.c. distilled water, injected into dorsal lymph sac.

12.41 p.m. Frog executes one or two leaps, which appear unusually high, and croaks while jumping.

12.42 p.m. Flexion of hind limbs performed slowly. Frog, in alighting after a leap, fails to support itself on its fore legs and falls flat on the table. Respiration rather feeble.

12.43 p.m. Jumps feebly, hind limbs being obviously trailed after the jump, and only slowly drawn up again.

12.44 p.m. Progresses by crawling in preference to jumping. Placed on back does not attempt to turn over. Respiration slow but vigorous.

12.52 p.m. Turns over when placed on back. Respiration more rapid. Flexion of hind limbs still markedly defective. Killed by pithing.

After doses up to 5 mgms. the frog gradually recovered during the succeeding two or three days. No attempt was made to determine the lethal dose. A prominent feature in the effect was the ease with which the animal became fatigued. After 2 to 3 mgms. the frog could for some time execute a fairly powerful leap, with prolonged extension of the hind limbs. A few such leaps, however, performed at intervals of a few seconds in response to irritation of the circumanal skin, exhausted the animal, so that no further leaps could be executed until after a rest of several minutes. That this fatigue was in part peripheral was shown by the records obtained with muscle-nerve preparations from such frogs. Even the first twitch-curve recorded after preparation showed considerable prolongation, especially of the last part of the relaxation. These peculiarities were accentuated by giving a series of stimuli at intervals of a few seconds. The great prolongation of the last part of the relaxation is shown very markedly in a fatigue-record taken with a slowly moving drum and stimuli at intervals of two seconds. The second stimulus occurs long before the lever has returned to the base line, so that the first five or six twitches are performed with increasing internal support. The muscle behaves, in fact, very similarly to one already exposed to fatigue.

Since this effect had some features in common with the veratrine-like action attributed to cornutine by Kobert, who found that, with large doses, the preliminary excitant effect might be overshadowed or lost altogether, an attempt was made to produce the true cornutine convulsions by giving very small doses of ergotoxine; $\frac{1}{10}$, $\frac{1}{20}$, and $\frac{1}{30}$ mgm. of ergotoxine phosphate, injected into frogs of 17, 12, and 30 grammes respectively, produced only the same symptoms as the larger doses in a weak and evanescent form. There seems no room for doubt, therefore, that, while the cornutine of Kobert may have contained, indeed, almost certainly did contain, some ergotoxine, the most characteristic of its effects on the frog must have been due to some other constituent.

The effects of ergotoxine on mammals, when injected intravenously or intramuscularly, presented a rather greater resemblance to the cornutine effects described by Kobert. In the rabbit the effects varied considerably in intensity in different individuals, and it was not possible to assign any accurate value to the lethal dose. The following experimental records indicate the general course of the action :—

(1) Rabbit, 1790 grammes.

12.10 p.m 3 mgms. of ergotoxine phosphate in 1·5 c.c. of distilled water injected into lateral vein of the right ear.

12.11 p.m. Ears very pale. Jerky movements of the legs.

12.15 p.m. Ears no longer pale. Restless, jerky movements continue.

12.18 p.m. Marked tendency to sprawl on the table with legs outspread. Head depressed. Feet seem to slide away from the animal till it sprawls flat, when there is a sudden, jerky recovery of the more normal crouching attitude. This is repeated frequently.

12.31 p.m. Twitching of ears and eyelids, teeth chatter.

12.35 p m. Much mucous secretion in nose, and saliva in mouth. Noisy breathing.

12.37 p.m. Legs completely extended on table: occasional convulsive movements. Rabbit has frequently, since the injection, passed moist faecal pellets.

12.46 p.m. Animal feels hot. Temperature taken in rectum and found to be 44° C.

12.49 p.m. Respiration ceases.

12.50 p.m. Thorax opened. Heart still faintly beating. Right ventricle greatly distended. No clots in heart or great vessels.

The high temperature at and after death seemed to be a characteristic effect of fatal doses. In another rabbit of 2950 grammes, an intravenous injection of 10 mgms. of the

NOT TO BE TAKEN AWAY.

pure alkaloid caused death in 1 hour 25 minutes with post-mortem temperature of 44·5° C. It is of interest to mention, in this connexion, that Zutz (36), with a commercial specimen of 'Cornutin citrate,' observed no rise of temperature in a guinea-pig, which, after an injection of 10 mgms., recovered in 1½ hours.

(2) Rabbit, 1410 grammes.

12.4 p.m. 1 mgm. of ergotoxine phosphate dissolved in 0·5 c.c. of distilled water injected into right ear-vein.

12.12 p m. No symptoms except very rapid respiration and prominence of the eye-balls.

1.10 p.m. Respiration still rapid. Jerky movements begin.

2.0 p.m. Recovered.

(3). Rabbit, 1420 grammes.

11.55 a.m. 2 mgms. ergotoxine, dissolved in 1 c.c. dilute NaOH, injected into right ear-vein, 2 mgms. similarly into left ear-vein. Altogether, therefore, 4 mgms.

11.58 a.m. Very rapid respiration and twitching of the limbs.

12.5 p.m. Fore-legs unable to support the animal, which sprawls with its nose on the ground. Persistent fine tremors and occasional twitching of the limbs.

12.42 p.m. All legs extended. Dyspnoea continues.

12.56 p.m. Rectal temperature, 42° C.

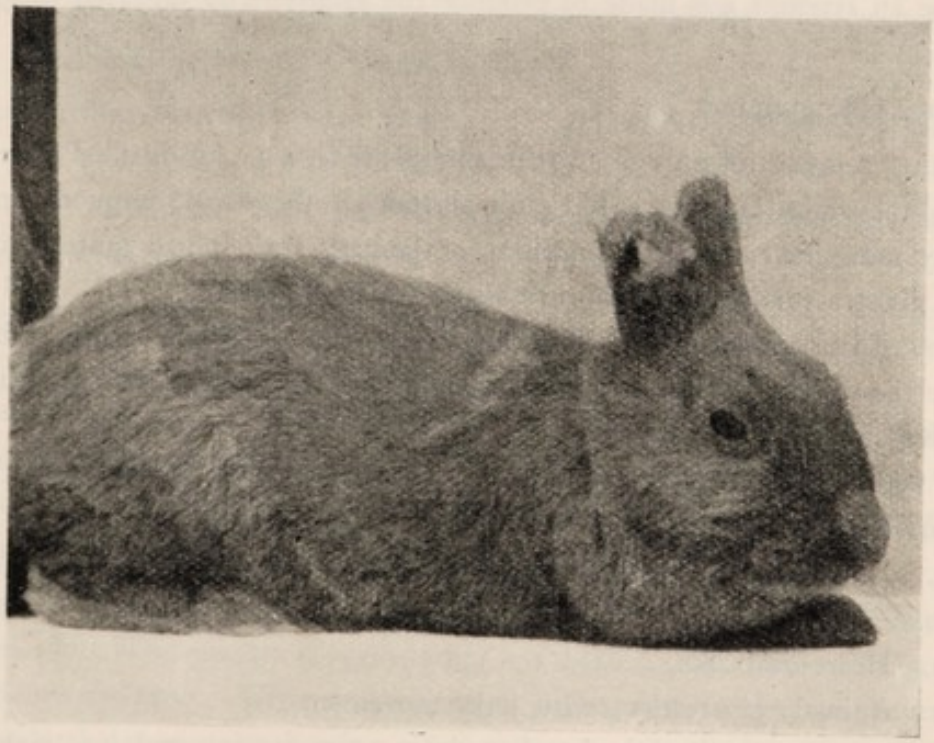


Fig. 5

Rabbit, showing ears shortened by gangrene two months after injection of 4 mgms. of ergotoxine.

From this time, until recovery begins, animal lies with fore limbs spread wide and hind limbs extended. The latter are occasionally and alternately flexed and again extended, producing a wobbling of the hinder part of the animal on the fore part, which remains stationary, supported on the broad base formed by the chest and the spread fore limbs. The head is at first depressed, with nose on the table: later (2.30 p.m.) it is raised. At 3.30 p.m. profuse salivation was noticed, saliva dropping from the mouth on to the floor. Respiratory sounds indicated bronchial secretion. About 5 p.m. recovery began. Animal could now draw up the hind legs and the dyspnoea was less severe. Next morning recovery was practically complete, and the animal remained apparently normal for a fortnight. It was then noticed that the skin of the peripheral two-thirds of each ear was becoming darker in colour, greasy in appearance, and losing its hair. A week later a distinct, sinuous line of demarcation separated the healthy proximal from the now obviously gangrenous peripheral portions. These peripheral portions gradually dried and were ultimately shed, without any bleeding, a month after the first appearance of the gangrene, and therefore six weeks after the single injection. A photograph, reproduced in Fig. 5, was taken, and the rabbit then killed and the head preserved.

This occurrence of true gangrene in the rabbit, after administration of an ergot-preparation, appears to be unique: we have, at any rate, been unable to find another case in the literature, though Kobert mentions the occurrence of subcutaneous bleeding in a rabbit's ear as a result of sphacelinic acid. Nor have any of the other rabbits which received sub-fatal doses of ergotoxine in our experiments shown any trace of such an effect. In one case an attempt was made to produce the gangrene by repeated sub-fatal doses as follows:—

(4) Rabbit, 1550 grammes.

January 9th. 2 mgms. of pure ergotoxine phosphate in 1 c.c. of distilled water, injected into the right ear-vein. The animal showed the usual dyspnoea and restless, jerky movements. On the morning of January 10th it had quite recovered and was given a further injection of 2 mgms. intraperitoneally. The usual symptoms followed in a milder form. The animal was then left till February 15th. By this date the weight had risen to 1900 grammes and the animal seemed quite well and showed no trace of gangrene.

February 15th. Weight 1900 grammes. Temperature 38·7 Seems perfectly normal. Heart-beat 300 per minute.

11.40 a.m. 2 mgms. ergotoxine phosphate in 1 c.c. distilled water injected into left ear-vein.

11.41 a.m. Heart-beat 280.

11.42 a.m. Animal very restless, with jerky movements.

11.47 a.m. 2 mgms. ergotoxine phosphate in 1 c.c. water injected into right ear-vein. Injection immediately followed by curious movements, the hinder part of the animal raised on the rather rigid hind limbs, while the fore feet are flexed so that the animal is supported on the wrists.

11.51 a.m. Heart-beat 160 per minute. Animal now holds itself high off the table, the body being moved restlessly from side to side and the muzzle depressed between the fore limbs.

12.0 noon. Restless movements continue: the characteristic sliding sprawl of the fore limbs, with jerky recovery, has appeared. The advanced position of the hind feet leads to a gradual movement backwards to the edge of the table.

1.30 p.m. The restless movements have almost vanished. The animal appears to be hyper-excitable, a slight touch on the back eliciting a sharp contraction of all the muscles of the body.

2.10 p.m. 20 c.c. dark coloured turbid urine passed. No albumen.

2.45 p.m. Less excitable. Fore legs still sprawl, so that chest lies on the table. Head raised. Respiration very rapid (340 per minute), and so vigorous as to shake the whole animal. Temperature 40·9° C.

2.50 p.m. Marked weakness, animal lying with all limbs extended. Moist faecal pellets passed. Anal sphincter apparently quite competent.

February 16th. Animal seems rather weak, but otherwise normal. Faeces still moist.

12.19 p.m. Heart beat 280 per minute. Respiration 100 per minute. 2 mgms. of ergotoxine phosphate in 1 c.c. distilled water injected into left ear-vein. Heart-beat irregular. Characteristic position and movements.

12.27 p.m. Injection of 2 mgms. similarly into right ear-vein. Rigidity of hind limbs and jerky movements intensified. Heart-beat 100 per minute. Respiration very rapid and shallow with marked nasal movements: could not be accurately counted.

2.45 p.m. Only slight hyper-excitability remained.

Several further injections were given, with diminishing severity of result. The following were the doses and other details:—

February 18th. 4 mgms. Salivation noticed for the first time with this rabbit.

February 19th. 5 mgms.

February 20th. 5·5 mgms.

February 21st. 5 mgms.

February 22nd. 10 mgms. The resultant nervous symptoms were not more severe than those originally produced by 4 mgms. On February 23rd the weight, which had been practically constant at 1900 grammes since February 15th, had risen to 1920 grammes, and by February 25th again to 2000 grammes.

February 25th. 15 mgms. in all injected, 10 mgms. into the ear-veins, 5 mgms. hypodermically. The effect was again rather less marked than that caused by 10 mgms. on February 22nd. With each injection subsequent to that on February 15th the animal passed normal urine a few minutes after the injection. This animal is still (April 2nd) under observation, and up to the present has shown no signs of the development of gangrene or any other bad effect as the result of the repeated injections, amounting in all to 56·5 mgms. of the pure phosphate.

There is clear evidence in this last case of the development of tolerance with repeated administration of the alkaloid. As already indicated, the reaction of different individuals showed variation, but intravenous injections of 5 mgms. per kilo. body-weight, such as were ultimately borne by this rabbit with only temporary symptoms of intoxication, have, in our experience, always proved fatal within two hours, if given as an initial dose.

The symptoms exhibited by the cat, when ergotoxine was given intramuscularly to the intact animal, were, as might have been expected, more striking and characteristic than those seen in the rabbit. The following are records of the three experiments made :—

(1) Male Cat, 3450 grammes.

12.26 p.m. 5 mgms. of ergotoxine phosphate in distilled water injected into the thigh muscles.

12.32 p.m. Cat seems unwell. Mews repeatedly.

12.33 p.m. Vomits.

12.35 p.m. Obviously ataxic and uncertain in its gait.

12.38 p.m. Unable to stand : falls if placed on its feet.

12.44 p.m. Lies on one side with the head raised from the floor. Occasionally moves the head and fore limbs. If placed on its feet at once rolls over.

12.56 p.m. Lies quiet if not touched, but, if touched, is evidently hyper-excitable, starting violently.

1.4 p.m. Marked salivation.

1.15 p.m. Becoming drowsy.

1.16 p.m. Salivation very profuse, watery saliva dropping from the mouth. Pupils becoming constricted.

2.0 p.m. Respiration 240 per minute. Mouth open ; salivation continues.

2.30 p.m. Pupils are now intensely constricted, and no longer dilate at all when the eyes are shaded.

2.50 p.m. Rectal temperature 42° C.

4.0 p.m. Lies on its side still, but can just stand unsteadily if placed on its feet. Right pupil still minimal ; left dilates slightly in the dark. Thin slime passed continually from the anus. Obvious paralysis of the internal anal sphincter, the lightest pressure on the abdomen causing the passage of slimy semi-fluid faecal matter. Firmer pressure needed to express the urine, the urethral sphincter being more competent.

6.0 p.m. Cat seems better and holds up its head. Pupils now dilate slightly in the dark. Respiration deeper and slower—136 per minute. Heart-beats very powerful —167 per minute. Anal sphincter still incompetent. Rectal temperature 41·5° C.

The cat was found dead at 8.30 next morning. The lungs were found very greatly congested; the abdominal lymphatics were very red, as was also the suprarenal medulla.

(2) Large Female Cat in Advanced Stage (Last Week) of Pregnancy.

August 23rd.

2.42 p.m. 3 mgms. of ergotoxine phosphate in distilled water injected into the thigh muscles. Cat rapidly became sleepy and lethargic, but otherwise showed nothing abnormal at first.

3.11 p.m. Passed a large volume of urine.

3.18 p.m. The large horns of the uterus, which had previously felt soft, could be felt, through the abdominal wall, to be hardening round the embryos.

3.30 p.m. Pupils are now contracted. Cat lying down and very sleepy. Uterus feels very hard and tightly contracted round the foetal sacs, which have been pushed tailwards by the contraction.

3.45 p.m. Uterus still tightly contracted. Cat rises to its feet, but soon sinks back on to the floor.

5.0 p.m. Condition the same, except that the pupils now dilate slightly when shaded.

August 24th.

8.0 a.m. The cat had already borne two dead, almost fully developed kittens; at 8.30 a.m. a third was born without difficulty, and found to be also dead. The placenta was in all cases expelled normally and without haemorrhage. Cat very sleepy all day.

August 25th. Cat quite well and moving about normally.

(3) Female Cat, 3420 Grammes, About the Middle of Pregnancy.

August 24th.

10.30 a.m. 3 mgms. of ergotoxine phosphate in distilled water injected into the muscle of the thigh.

10.35 a.m. Respiration shallow and irregular. Commencing ataxia.

10.45 a.m. Respiration more rapid. Cat is markedly ataxic, and also hyper-excitable, jumping and spitting if touched lightly.

10.50 a.m. Profuse salivation, saliva dropping from the mouth.

11.0 a.m. Sphincter and internus paralysed, slimy faeces being expressed by slight pressure on the abdomen.

2.0 p.m. Uterus obviously contracted. Sphincter of pupil constricted.

5.0 p.m. Uterus now feels hard and tightly constricted round the foetuses. The other symptoms passing off.

August 25th.

9.30 a.m. Blood-stained watery fluid has been passed from vaginal orifice, and is still being passed slowly. No foetuses in the cage. Abdominal palpation reveals no uterine horn on the right side, where formerly it was felt with two foetuses. On left side a large, soft, elongated mass can be felt. Cat seems fairly well.

August 27th. Condition still the same. Blood-stained fluid still trickling from the vagina. Cat seemed unwell, and was killed by chloroform.

On post-mortem examination all organs seemed normal except the uterus. Of this both horns were found on the left side of the animal. They were somewhat soft and 'boggy,' and blood-stained. The right horn contained two foetuses in a common bag of membranes. The amniotic fluid was blood-stained, and the foetuses, dark red and very soft, had obviously been dead for some time. The placentae were easily separable, soft and friable, and chocolate-coloured. The vagina was normal.

The fact being thus established that ergotoxine causes, in the intact pregnant cat, a tonic contraction of the uterus, we did not consider it worth while to pursue this particular line of experiment further. Several observers have laid much stress on the regular production of abortion as an essential characteristic of the active therapeutic principle of ergot. The claim of the ergot alkaloids to be regarded as such therapeutic principles has recently been adversely criticised by Kraft from this point of view. But, as has already been pointed out elsewhere—Barger and Dale (47)—the experiments recorded in his paper were made on rodents, which are relatively very insensitive to the action of ergotoxine. Moreover, in any case, there seems little warrant in the historical accounts of epidemics of ergotism, in medico-legal records, or in the practical use of ergot in obstetrics, for expecting of the active principle that it shall, in all cases, and at any period of pregnancy, produce abortion. In the four cases described by Kobert (20) of pregnant women who had taken ergot with the object of inducing abortion, death in all cases supervened before abortion had occurred, and in only one case had the expulsion of the foetus even begun.

Historical evidence seems to us to be in no way opposed to the view that abortion, following ergot-ingestion, is due to the production of a tonic uterine contraction, leading to asphyxiation of the embryo, followed, in the natural course, by its expulsion. We consider it sufficient, therefore, to have shown that large doses of ergotoxine can produce contraction of the pregnant uterus, at a time when such contraction is opposed to the natural, physiological tendency of the organ. That the foetuses, killed by the uterine tonus, may be, in some cases, retained days after the immediate effect of the drug has

passed off, seems to us to be of little consequence. The important conclusion, from the practical point of view, is that the alkaloid, in doses too small to cause general symptoms, might be expected to reinforce the natural tendency of the uterus to tonic contraction *post partum*. Whether that conclusion is justified must be determined by clinical experience.

The domestic fowl has been more frequently, perhaps, the subject of experiment in investigations on ergot than any other animal. It was of particular interest and importance, therefore, to determine the action of ergotoxine on the cock.

Kobert, finding that cornutine, like sphacelinic acid, caused a rise of blood-pressure, was surprised at the failure of the alkaloid to cause gangrene. On the other hand, he found that 4 mgms. of the alkaloid, given hypodermically to a cock of 2½ kilos., caused narcosis, and, about two and a half hours after the injection, death in convulsions.

This marked convulsant effect on the fowl, like the similar effect on the frog, was not, in our experience, obtained with ergotoxine. The effect of this alkaloid, indeed, showed considerable variations with different individuals. Although our attention was not deliberately directed to the point, and though no attempt was made to use thoroughbred specimens, we got the impression that there are considerable differences in the reaction of different breeds of fowls. The following are typical examples of a number of experimental records[1] :—

(1) Black Cockerel, with bright red comb.

July 18th.

12.26 p.m. 20 mgms. of ergotoxine phosphate in 5 c.c. distilled water injected into the breast muscle.

12.30 p.m. Comb becoming paler, especially at the root. Skin round the eyes quite pale.

12.31 p.m. Beak open : breathing quick and laboured.

12.45 p.m. Comb still pale over the eyes : tips rather darker, and bluish in tinge. Wattles pale and cold. Condition continued much the same during the afternoon.

1. The injections were kindly performed by Mr. C. T. Symons.

5.15 p.m. Skin round the eyes and the root of the comb are still pale. Elsewhere the comb is of a dusky purple colour, mottled with redder patches. The bird feels hot. Temperature under the leg 43° C.; in the cloaca 44·5° C.

July 19th.

10.0 a.m. Comb red, except for a rather sharply-defined, blackish-purple area at the hinder margin, and the tips of the two last digitations, which are also blackish. The cock seems fairly well and walks normally

5.36 p.m. 10 mgms. of ergotoxine in 2·5 c.c. of dilute NaOH injected into the wing-vein.

6.0 p.m. Symptoms similar to those after the injection on the previous day.

July 20th.

11.0 a.m. The whole of the comb bluish-red in colour, darker at the tips of the digitations. Just over the eyes a portion is of the normal red colour. The cock breathes slowly with closed beak, and seems very drowsy, the eyes being closed except when the bird is roused. It squats on the ground, and, if dropped one foot to the floor, stumbles in alighting.

2.45 p.m. Just living but collapsed. Respiration slow and regular. Comb darker purple and cold.

2.50 p.m. A few convulsions, leading to death.

Post-mortem.—The skin over the breast was found discoloured, being green and dark-red in patches. The peritoneal cavity contained a green offensive-smelling fluid. The whole of the small intestine was congested, and the mucous membrane reddened, in places haemorrhagic and ulcerated. At the tip of the duodenal loop was an oval perforation, about 1 cm. in length, obviously the result of ulceration. The comb was dusky purple in colour, but had not in any part become dry and shrivelled.

(2) Small Black Cock (Cross-bred Minorca), with thin, erect comb; weight 1700 grammes.

July 18th.

3.5 p.m. 40 mgms. of ergotoxine phosphate, in a gelatin capsule, given by the mouth.

4.0 p.m. Hinder part of the comb seems slightly darker, the rest slightly paler than before. The effect is not at all pronounced, and is accompanied by no other abnormal symptoms.

5.45 p.m. Condition unchanged.

July 19th.

9.30 a.m. The cock is normal. The hinder end of the comb appears a trifle bluish, but not more so than it often appears in normal birds.

3.3 p.m. 7 mgms. of ergotoxine phosphate, dissolved in distilled water, injected into the wing-veins.

3.5 p.m. The root of the comb pale, as is also the skin round the eyes. Head depressed. Wings drooping. Beak open. Some salivation. Slight dyspnoea. This condition continued for the rest of the afternoon.

July 20th.

11.0 a.m. The tips and hinder part of the comb are quite dark, especially the small papillae. The flat expansion behind the last digitation is bluish in colour. The rest is normally red, but feels cold. The lower part of the wattles is dusky and cold.

5.30 p.m. Cock rather sleepy. Comb unchanged.

July 21st. The whole comb red again except hinder edge and tips of last two digitations. Cock seems well and vigorous.

11.7 a.m. 10 mgms. of ergotoxine phosphate in distilled water injected, 5 mgms. hypodermically into the breast, 5 mgms. intravenously into the jugular vein.

Effects as before appearing a few minutes after injection, whole comb becoming dusky purple, except a pale area at the root continuous with the pale skin round the eyes. Constitutional symptoms similar to those following previous injection.

4.0 p.m. Ataxia, dyspnoea, and prostration have disappeared, the cock strutting about normally. Comb red, except the hinder part, which is still purple, the tips of the two last digitations being black.

July 23rd.

10.0 a.m. Whole comb bright red and warm, except a narrow band round hindmost flat expansion and the tips of the two hindmost triangular digitations. These parts are all black, dry, slightly shrivelled, and quite insensitive. The lower edge of the left wattle shows the same condition.

12.5 p.m. 10 mgms. of ergotoxine in 4 c.c. dilute NaOH injected into the breast muscle. The comb showed the usual changes. The constitutional symptoms were less marked than with former injections, salivation being the most prominent.

July 24th. Cock seems fairly well. Weight has fallen only to 1620 grammes. The black area at the hinder end of the comb is more extensive. 10 mgms. of ergotoxine in dilute NaOH again injected into the breast muscle, the usual changes in the comb following, but the constitutional effects being again less severe.

July 25th. Still further extension of gangrene. Otherwise the bird seems normal. 10 mgms. of ergotoxine phosphate in distilled water injected under the skin of the neck. The usual changes in the comb appeared, developing rather slowly. Constitutional effects slight.

July 26th. Comb still purple, except at the root, where it is pale. Intense pallor of the skin round the eyes. The gangrenous process is extending. The wattles are pale at the root. Below they are swollen and purple, and the skin is peeling (moist gangrene).

July 27th. Most of the comb again red. The black area at the hinder end is now broad and sharply demarcated. The dry gangrene of the two last digitations is advancing. The lower ends of both wattles show the moist gangrenous condition, and, continuous with this, a broad flat tip of dry gangrene on the left hand side. Cock weighs 1470 grammes.

The bird was kept under observation. The weight fell to 1300 grammes on July 30th, and then increased to 1450 grammes again by August 2nd. On August 9th the condition was unchanged, except that the line of separation of the gangrenous portions was becoming gradually more definite. A further injection of 10 mgms. of ergotoxine was given into the breast muscle. The usual comb-changes were produced in a slighter form, and by August 10th the bird was as before.

On August 11th the gangrenous portion of the lower end of the wattle was found to have fallen off. The line of separation was perfectly healed, and there had been no bleeding. The tips of the digitations and the area at the hinder end of the comb separated on September 1st and 13th respectively. The abbreviation of the comb and wattles remained very evident till the spring, when the loss was rapidly repaired.

The following is an instance of acute intoxication :—

(3) White Cock, weighing about 2 kilos. Comb erect and scarlet. Skin round the eyes and wattles also bright scarlet.

1.11 p.m. 5 mgms. of ergotoxine phosphate in 2.5 c.c. of distilled water injected into the left wing-vein.

1.12 p.m. 4 mgms. similarly injected into the right wing-vein. There was immediate ataxia, and simultaneously the skin round the eye and the root of the comb became pale, the rest of the comb darkening.

1.14 p.m. Intense ataxia. Head held down on the table with open beak, wings drooped ; bird cannot support itself on its legs.

1.19 p.m. Whole comb and wattles very dark purple. The tips of the digitations and the hinder end of the comb are especially dark. Very marked dyspnoea as well as ataxia. The prostration gradually advanced, the breathing becoming more feeble till at 2.1 p.m. it stopped.

Post-mortem Examination at 3.0 showed some congestion of the bowels. The right auricle and ventricle, and also the left auricle, contained large clots. The left ventricle was quite empty. The lungs congested, but less so than was expected.

A number of attempts to induce gangrene failed, owing to the death of the bird when the effect was just beginning. Although the dose given by the mouth to the cock in Experiment (2) had been without obvious effect, it was thought that it might be connected with the ultimate successful production of gangrene in that experiment. Another cock was, therefore, given large doses (in all 80 mgms.) of ergotoxine by the mouth, and, when this had proved without effect, subsequent

intravenous and intramuscular injections. The usual temporary discoloration of the comb, attended by ataxia and dyspnoea, were produced, but no true gangrene followed.

The following experiment, undertaken with the idea of producing the gangrene by repeated small doses, is chiefly of interest as indicating the severe effects of even small doses of the pure alkaloid :—

(4) Cock, with Speckled Plumage, very large erect red comb and large pendulous wattles. Weight 1770 grammes. 10 mgms. of the phosphate of ergotoxine, purified by repeated recrystallisation, were dissolved in 0·5 c.c. of absolute alcohol, four drops of 10 per cent. NaOH added, and the solution then made up with water to 5 c.c.

3.1 p.m. 1 c.c. of this solution, containing ergotoxine corresponding to 2 mgms. of the phosphate, injected into the left wing-vein. The skin round the eye immediately became pale.

3.2 p.m. Wattles also very pale. Bird crows loudly and walks about the room without ataxia.

3.4 p.m. Wattles livid. Pallor spreading to the root of the comb.

3.5 p.m. Gait becoming ataxic.

3.10 p.m. Ataxia very marked. Head drooped forward, beak open, and salivation profuse. Whole comb, with the exception of a patch at the front end, dusky purple in colour.

3.25 p.m. Ataxia has practically disappeared, the bird being now only sleepy. Root of the comb is pale, the rest dusky purple, the darkest portions being, as usual, the hinder flat expansion, the tips of the last three digitations and the lower ends of the wattles.

4.40 p.m. The front part of the comb is recovering its normal colour in patches.

4.42 p.m. Another 2 mgms. injected into the left wing-vein. The symptoms were reproduced, but the ataxia was more evanescent. Salivation again marked.

5.18 p.m. 6 mgms. in 3 c.c. injected into the breast muscle. The ensuing ataxia was less pronounced, but more persistent, being quite perceptible on the following morning, as was also the discoloration of the whole comb, and the excessive salivary secretion.

At 10.24 on the next morning a further injection of 4 mgms., similarly dissolved, was made into the breast-vein. By 12.15 the ataxia resulting had nearly disappeared, and salivation had ceased. The comb presented the dusky colour, the intensity of the change having the usual distribution.

At 12.59 p.m. 3 mgms. of ergotoxine were given intravenously. The ataxia quickly passed off. At 6 p.m. the hinder end of the comb and the digitations were very dark. The bird seemed sleepy, but otherwise not unwell.

On the following day the bird was found dead in its cage at 3 p.m. Post-mortem the only abnormality found was an inflamed condition, acute in patches, of the mucous

membrane of the whole intestinal tract. The proventriculus showed follicular catarrh. The crop was not noticeably affected. The comb was pale, but fairly normal in colour, except for thin black strips at the anterior and posterior ends, and the black tips of the digitations.

It was evident that this administration of 17 mgms., spread over two days, was still too rapid to give time for the development of gangrene before the death of the animal. It was thought that absorption should take place more slowly if the free alkaloid were dissolved in alcohol and given hypodermically. The insoluble base, precipitated as the alcohol became mixed with the tissue fluids, should, it was thought, be slowly absorbed, time being thus given for the development of a more chronic action. That this expectation was justified is shown by the following experiment :—

Black Cock, weighing 2120 grammes, with large bright red comb.

100 mgms. of pure ergotoxine oxalate was dissolved in 3 c.c. of absolute alcohol. To this was added an equivalent of Na_2CO_3 in distilled water. The precipitate of sodium oxalate was filtered off. The filtrate was made up to 10 c.c. with absolute alcohol, thus giving a solution of the free alkaloid in 80 per cent. alcohol.

September 17th.

3.20 p.m. 1 c.c. of the above alcoholic solution, containing 10 mgms. of free base, injected under the skin of the breast.

3.22 p.m. Skin round the eye becoming slightly pale. The comb became very slowly pale at the root and dusky elsewhere. By 6.0 p.m. the duskiness of the comb was marked, but the bird seemed quite well and vigorous.

September 18th.

11.15 a.m. The whole comb was still rather dark in colour, the tips of all the digitations being black.

11.33 a.m. A further 1 c.c. of the same alcoholic solution of ergotoxine injected hypodermically. The onset of the effect was again gradual. By 5.15 p.m. the whole comb and wattles were blackish purple in colour, except two small red patches in the middle of the comb.

September 19th.

11.30 a.m. The body of the comb is again red, with black patches. The skin round the eyes is still rather pallid. *All the digitations are black*, and their tips are beginning to dry. The bird seems fairly well, and shows no sign of ataxia. Weight 2070 grammes.

September 20th.

10.45 a.m. Weight 2080 grammes. All the comb has regained its natural colour, with the exception of the digitations which are all intensely black, and drying at the tips, as much as 0·5 cm. being dry and shrivelled.

10.55 a.m. Hypodermic injection of a further 10 mgms. of ergotoxine in 80 per cent. alcohol. The effects were again similar, and the gangrenous process was found to have advanced on the 21st, when a further 10 mgms. were injected. After this last injection the hinder part of the comb remained dark in colour till the 25th, when 20 mgms. were injected in similar solution. The whole comb then remained dark purple for forty-eight hours, when the effect again gradually receded, leaving practically the whole of the part of the comb behind the root black and drying. This, with the last three digitations, was shed in one piece about a fortnight later. The tips of the digitations anterior to this were similarly separated.

Fig. 6 shows a photograph of this bird taken in the following March, when the comb was beginning to grow again.

FIG. 6

Cock, with comb reduced by gangrene (see text). The shape of the comb before the experiment is roughly outlined.

Throughout this experiment the bird exhibited no well-developed ataxia, no disorder of the digestive tract, and apart from the effect on the comb, remained practically in normal health.

The resemblance between the effects of this administration of ergotoxine, by a method ensuring slow absorption, to those obtained by Kobert with sphacelinic acid given in initially small but gradually increasing doses, is very striking. The effects in the earlier experiments, on the other hand, when the ergotoxine was injected in water-soluble form, are more reminiscent of those which he obtained with large initial doses of sphacelinic acid.

It follows that Kobert's conclusions as to the part played by sphacelinic acid in the epidemics of ergotism can, for the most part, at any rate, be transferred to ergotoxine. We are not at present in a position to discuss the conditions which determine the absorption of ergotoxine from the alimentary canal when it is mixed with other substances, as in sphacelinic acid or native ergot, whereas an equivalent amount of a pure ergotoxine salt is practically without effect when given by the mouth. It is less difficult to interpret the powerful action of the pure salts of ergotoxine given hypodermically as compared with its comparatively feeble effect when injected in a resinous mixture such as sphacelinic acid (Kobert). There is no room for doubt, however, that under conditions which ensure its satisfactory but not too rapid absorption ergotoxine is the cause of the gangrenous type of ergotism. On the other hand, its relation to the convulsive nervous type is less clear. Nervous symptoms, indeed, are prominent in acute poisoning by ergotoxine injected intravenously; but with slow administration we have never seen anything suggestive of convulsive ergotism, in which it is possible that, as Kobert suggested, a different active principle is concerned. Of particular interest in connection with the action of ergotoxine are certain diseases which have been mentioned as possibly due to ergot poisoning (see Ehlers (34)). One of such is Raynaud's symmetrical gangrene, which, it has been suggested, may be a sporadically occurring delayed ergotism. The case of the one rabbit, in which we observed gangrene some weeks after a single non-fatal injection of ergotoxine, is an interesting parallel.

Still more interesting, in view of our description of sympathetic motor (including vaso-motor) paralysis as the most characteristic

effect of ergotoxine, is the connection, suggested by Ehlers, between ergot and the disease described by Weir Mitchell as erythromelalgia, in which vaso-motor paralysis in the extremities is the most prominent symptom.

The Relation of Ergotoxine to Other Ergot Alkaloids

(*a*) *Hydro-Ergotinine*

In a private communication to us, Herr Kraft states his agreement with our conclusion that the alkaloid which he described as hydro-ergotinine is identical with our ergotoxine.

(*b*) *Ergotinine*[1]

As Tanret's crystalline ergotinine was the first well-defined alkaloid in ergot, it was, not unnaturally, assumed by its discoverer to be the active principle. Experiments on animals, made in France, to some extent seemed to support this view. Since Tanret, however, applied the name 'ergotinine' also to the amorphous mixture which contains the highly active 'ergotoxine,' it is obvious that no weight can be attached to experiments of which it is not definitely stated that the specimen used was wholly crystalline. On this ground the clinical results of Chahbazian, and the more recent physiological experiments of Plumier, must be set aside. On the other hand, Kobert, who used a pure specimen, consisting of 'very beautiful white needles,' prepared by Tanret himself, found only a trace of doubtful activity, 10 to 100 mgms. being without effect on frogs, while similar doses caused only a trifling rise of blood-pressure in mammals, without convulsions or other symptoms. Similarly Meulenhoff concluded that the activity of the crystalline ergotinine represented only a very small part of that of the ergot containing it.

The results of our own experiments point in the same direction. We have examined commercial specimens of ergotinine obtained from two well-known German firms. The specimens were described

1. Tanret distinguishes amorphous and crystalline ergotinine. It will be seen that we regard the so-called amorphous ergotinine as chemically different from the crystalline and as, in any case, impure. When, therefore, we use the term 'ergotinine' without qualification we mean the pure, crystalline alkaloid.

as crystalline, but contained at least 50 per cent. of an amorphous, greyish-green impurity, which was readily soluble in warm alcohol, and was strongly active physiologically. On recrystallising the part which was less readily soluble, from boiling alcohol, we obtained a rather small yield of white needles corresponding in all respects to Tanret's description of crystalline ergotinine.

The ergotinine thus obtained from the commercial specimens, and various other specimens of pure ergotinine prepared by ourselves, showed, on intravenous injection into cats, a very variable degree of activity. In two or three instances a complete vaso-motor reversal was obtained with doses of 1·5 to 2 mgms. per kilo. of body weight (as compared with 0·5 mgm. of ergotoxine). In most cases, however, a dose of 4 to 6 mgms. per kilo. produced only a slight rise of blood-pressure and a mere indication of the vaso-motor reversal; in a few cases there was no trace of either action. At first we were at a loss to explain these irregularities. All the specimens were well crystallised, and their purity was established as far as possible by analysis and by physical tests. We were led to the assumption that, according to the method used for putting the ergotinine into solution, a greater or smaller portion was decomposed, forming a highly active substance. Sometimes we dissolved the ergotinine in the minimum quantity of boiling alcohol (about 0·8 c.c. for 10 mgms.), added one or two drops of 10 per cent. caustic soda solution, and could then dilute with water. In other cases a little more than the theoretical quantity of phosphoric acid was added to the alcoholic ergotinine solution, which could then be diluted with water to a certain extent. We also dissolved the alkaloid in the minimum quantity of glacial acetic acid. Any attempt to neutralise the excess of acid, however, caused precipitation of the ergotinine salt.

After having found that a solution of ergotinine in caustic soda became somewhat more active on boiling, we investigated the action of phosphoric acid, hoping to produce the characteristic ergotoxine phosphate.

0·3 gramme of ergotinine was boiled for one hour with 10 c.c. of absolute alcohol, with a reflux condenser. As was expected, a considerable portion remained undissolved.

0·6 c.c. of 10 per cent. aqueous phosphoric acid solution, corresponding approximately to 1·2 molecular equivalents, was now added, and after five to ten minutes' further boiling solution was complete. On cooling, crystallisation was induced by scratching with a glass rod; the first crop of crystals weighed 0·07 gramme (= 25 per cent.), and consisted of minute diamond-shaped plates, melting at 182°. The thin prisms of ergotinine were entirely absent. As ergotoxine phosphate, crystallising in prisms, melts at 182° to 183°, and as the melting point was not lowered after mixing with a quantity of the new plates, it seemed probable that the latter represented ergotoxine phosphate. In support of this view was also the fact that the new substance yielded, on decomposition with ammonia, an amorphous base apparently identical with ergotoxine.

The new phosphate thus obtained from ergotinine was tested physiologically by intravenous injection into a cat. In the first place 3 mgms. of the same ergotinine specimen as that which had been submitted to hydrolysis were given, dissolved in 2·5 c.c. of 50 per cent. alcohol, mixed with a little saponin (to prevent crystallisation).

Then 2 mgms. of the new phosphate were injected, dissolved in a little dilute alcohol, to which the same quantity of saponin had been added. The effect of the two injections is shown in Fig. 7.

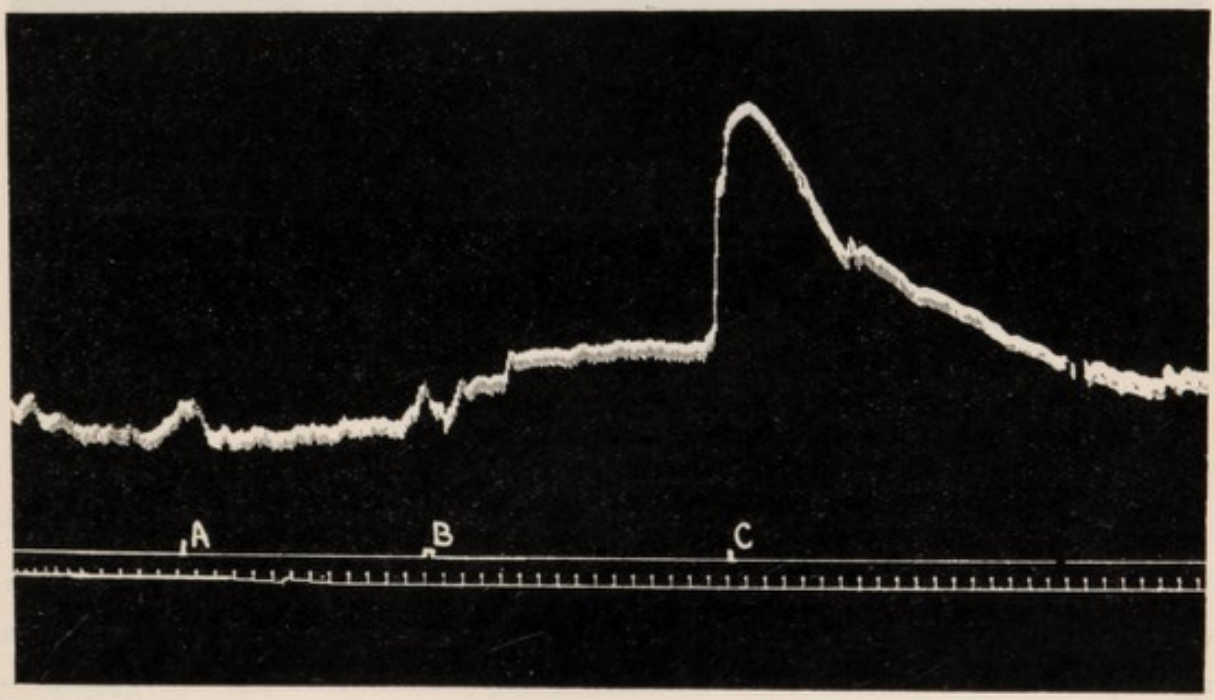


FIG. 7A

Cat, 2½ kilos. Pithed. Artificial respiration. Carotid blood-pressure. Injections into jugular vein.

Effects of ergotinine—

At A—1 mgm. of ergotinine.

At B—3 mgms. of ergotinine.

At C—0·05 mgm. of the suprarenal principle.

The ergotinine produces little pressor effect, and no subsequent vaso-motor reversal.

As will be seen, the ergotinine produced a small rise of blood-pressure, and practically no vaso-motor reversal, whereas a smaller quantity of the phosphate of the amorphous alkaloid, obtained from it, caused a very marked rise of blood-pressure and complete vaso-motor reversal.

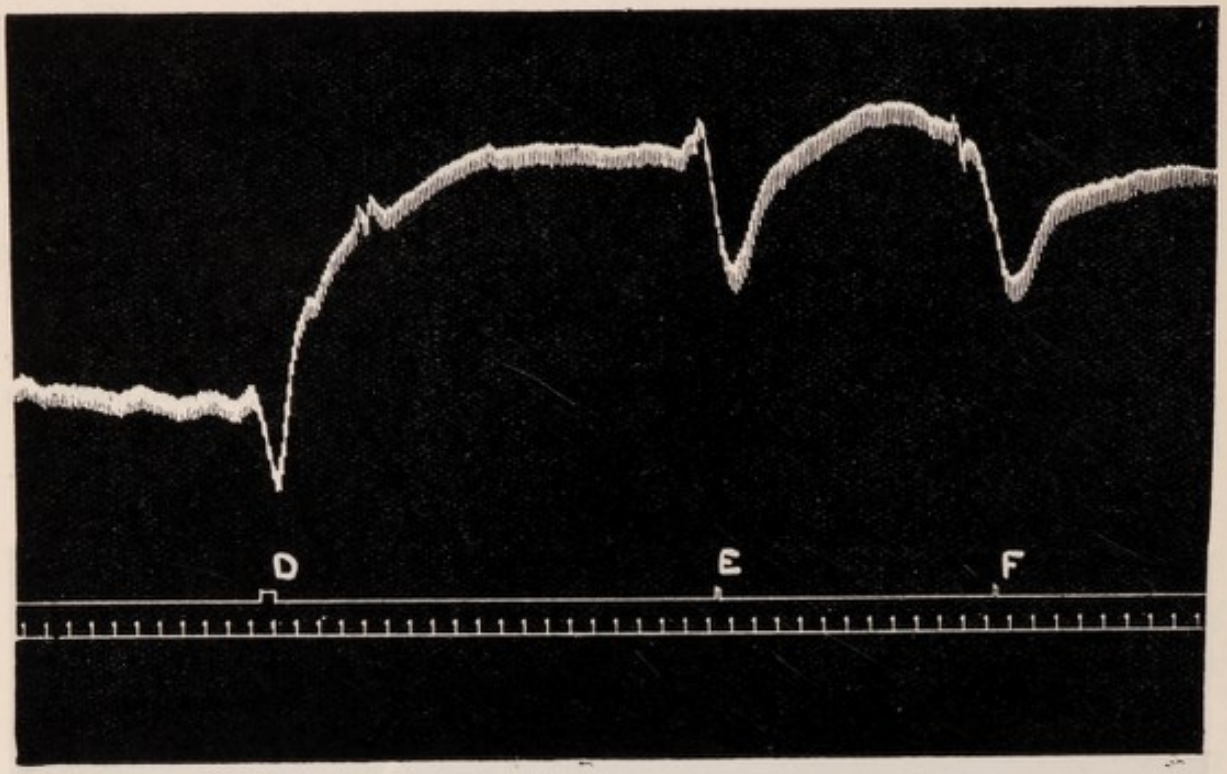


FIG. 7B

Same animal as in FIG. 7A

Subsequent effect of crystalline phosphate (ergotoxine)—
At D—2 mgms. of the crystalline phosphate.
At E—0·05 mgm. of the suprarenal principle.
At F—0·1 mgm. of the suprarenal principle.
The ergotoxine gives a good pressor effect and complete vaso-motor reversal.

We had previously obtained the same crystalline (ergotoxine) phosphate in an attempt to crystallise the phosphate of ergotinine. 1·15 grammes of ergotinine (crystallised from alcohol) was dissolved in a mixture of alcohol and ethyl acetate and 2·5 c.c. of 10 per cent. phosphoric acid were added. Accepting a wrong value for the molecular weight of ergotinine, we believed this quantity of acid to be equal to one molecular equivalent. In reality there was one and a quarter molecules. The solution of the phosphate was concentrated in a vacuum dessicator at 37°, and the amorphous substance which separated out (ergotinine phosphate ?) was filtered off. It was easily soluble in alcohol on warming, and on cooling it again separated out amorphous. Finally, however, on concentrating the mother liquor of the amorphous substance, we obtained a minute quantity of imperfectly formed

plates, which consisted of the phosphate of an amorphous base, and of which 1 mgm., dissolved in 0·5 c.c. of 50 per cent. alcohol produced complete vaso-motor reversal in a cat of 2·7 kilos.

This result, though somewhat puzzling at the time, is now perfectly intelligible in the light of our later knowledge.

These experiments make it clear that ergotinine is certainly much less active than ergotoxine, and make it, indeed, very doubtful whether as such it has any activity at all. It is true that the alkaloid, when separated in the pure condition, is a particularly unsuitable substance for physiological experiments. If, on the one hand, it is injected into the blood-stream in some neutral solvent, such as alcohol, the whole of the alkaloid separates in an insoluble form as soon as the solution comes into contact with the blood. If, on the other hand, it is dissolved for injection by the aid of acids or alkalies, not only does a considerable proportion of the feebly acidic and basic ergotinine separate out on dilution with the blood, but one cannot exclude, under such conditions, the possibility of ergotoxine formation. It may be conceded, therefore, that, if ergotinine could be brought into solution without risk of conversion into ergotoxine, and could remain dissolved in the body fluids, it might exhibit physiological activity, even when injected intravenously. If it is given hypodermically the possibility of slow conversion into ergotoxine in the subcutaneous tissues gives to ergotinine a further chance of showing itself physiologically active. This may, in part, explain the toxic effect of ergotinine on guinea-pigs, in the experiments recorded by Kraft. However that may be, our own experiments confirm those of Kobert and Meulenhoff, and lead to the conclusion that, whether because of its insolubility or otherwise, the activity of ergotinine is negligible in comparison with that of ergotoxine.

In support of this conclusion we may also cite Jacobj's experience (37) with the inactive alkaloid, which he named 'secaline,' but which can, beyond reasonable doubt, be identified with ergotinine.

Jacobj's reasons for regarding it as a new alkaloid were to a large extent imaginary, and founded on misconceptions. The first of these was as follows :—The alkaloid gave the colour reaction found by Keller to be characteristic of cornutine ; but it could not

be cornutine, which Kobert described as of great physiological activity, for Jacobj's alkaloid was physiologically inert, resembling in this respect the ergotinine of Tanret, which, on Kobert's authority, was devoid of activity. In other words, Jacobj's alkaloid resembled Tanret's ergotinine in its inactivity, but gave the colour reaction attributed by Keller to cornutine. The explanation is simple. The whole confusion is due to the fact that Keller, at the time when he described his reaction, regarded the two alkaloids as one, and selected for that one the name cornutine. On this ground, therefore, the evidence is all in favour of the identity of secaline and ergotinine.

Another of Jacobj's reasons for concluding against this identity was a chemical one. He found on analysis 49·01 per cent. of carbon and 11·62 per cent. of nitrogen, whereas Tanret had found in ergotinine 68·62 per cent. of carbon and 9·15 per cent. of nitrogen. The discrepancy in the nitrogen figures is explained by the fact that Tanret used the unreliable soda-lime method and that his figure was erroneous, as was shown by one of us in conjunction with F. H. Carr (51). The mean of four analyses according to Dumas gave 11·6 per cent. of nitrogen, a figure which Tanret himself has recently (49) confirmed. As will be seen, the agreement with the value given by Jacobj for secaline is excellent. We cannot explain the discrepancy of 20 per cent. between Jacobj's percentage of carbon and the figures found by Tanret and by Barger and Carr, but must be content to point out that Jacobj performed only one combustion, and that of an amorphous substance, the purity of which he deduced from its white colour and physiological inactivity. It is interesting to note that on repeating the preparation of secaline, Jacobj could not 'for some unknown reason' obtain a similarly inactive preparation. We may safely assume that this unknown reason was the presence of ergotoxine, which cannot be separated from ergotinine by precipitation of the ethereal solution with light petroleum, the method employed by Jacobj.

(*c*) *Cornutine*

In an earlier paper one of us (43) applied the name cornutine to an alkaloidal substance (which we now know to have been impure ergotoxine), on the ground that this substance resembled in solubility cornutine more than any other of the active substances hitherto described in ergot. We were anxious, moreover, to avoid 'the introduction of new names on the strength of physiological results, and in default of the chemical isolation of principles.' When this amorphous alkaloid had been obtained in a pure form it was found to differ not only in chemical properties from cornutine, but also in those physiological properties which, according to Kobert, constituted the real claim of cornutine to recognition as a separate substance. Among the few chemical characteristics of cornutine, which were

mentioned by Kobert, are insolubility in ether and the possession of a hydrochloride which was readily soluble in water. Our alkaloid is, like ergotinine, slightly but distinctly soluble in ether, and its hydrochloride is remarkably insoluble in water. From the physiological point of view, which is of primary importance in the case of a substance for which only 'physiological purity' is claimed, our alkaloid also showed very marked differences from cornutine. While resembling both sphacelinic acid and cornutine in causing rise of blood-pressure and contraction of the uterus, it differed from cornutine in both the properties which Kobert regarded as characteristic of that substance; it did not cause the strychnine-like spasms in frogs, and it did cause gangrene of the cock's comb. On the whole the physiological effect of the alkaloid was much more like that of sphacelinic acid than like that of cornutine. For a pure substance presenting such confusing resemblances to several 'active principles,' yet equally confusing differences from any one in particular, the choice of a new name was inevitable, and the name ergotoxine was accordingly suggested for it by Barger and Carr (45) in their chemical account of the substance.

There is, however, good reason for believing that cornutine contains some ergotoxine. According to Tanret (33), cornutine gives (feebly) the sulphuric acid colour reaction for ergotinine, which is also given, as we have pointed out, by ergotoxine. Specimens of cornutine, which we prepared according to Kobert's method, gave, on intravenous injection into cats, a considerable rise of blood-pressure, with some small subsequent indication of a vaso-motor reversal.

In addition to ergotoxine, it is probable that cornutine contains decomposition products of the former, and it is possible that these decomposition products are concerned in the strong convulsant action on frogs. It is noteworthy, however, that other observers, such as Meulenhoff, have failed to produce the convulsions in frogs with cornutine prepared according to Kobert's method. We may remark in passing that this method, which begins with an extraction of the ergot by dilute hydrochloric acid, is scarcely a suitable one for the

removal of the ergotinine and ergotoxine, since their chlorides are very little soluble in dilute hydrochloric acid, so that the yield of cornutine is necessarily very small.

As we have pointed out above, Kobert's term cornutine was applied later by Keller to ergotinine. 'Cornutin-Keller,' prepared by Keller and others, was examined physiologically by Santesson (39), who remarks that it is not identical with Kobert's cornutine, and that it is apparently less active. Santesson describes his preparations as impure; all but one were partially crystalline. Thirteen to twenty-five milligrammes injected hypodermically produced a violet coloration of the cock's comb, but true gangrene was never observed. In rabbits a dose of 10 to 16 mgms. per kilo., given hypodermically, produced no pronounced effect; 40 mgms. per kilo. caused a non-fatal intoxication. Intravenously 5 mgms. produced a marked rise of blood-pressure in the cock, but in rabbits only a small and transitory rise was occasionally obtained. Santesson inclines to the view that 'cornutin-Keller' is not the chief active principle in ergot, and that it is probably identical with the ergotinine of Tanret. From Santesson's physiological results and Keller's method of preparation it seems likely that the specimens employed were mixtures of ergotinine and ergotoxine, containing perhaps something like 25 per cent. of the latter alkaloid.

Santesson's failure to obtain a rise of blood-pressure in the rabbit in no way excludes the presence of ergotoxine in his preparations, for, as we have observed, rodents are especially insensitive to the stimulant action of ergotoxine, and in particular show but a slight vaso-constrictor effect. Moreover, his animals were not given artificial respiration, without which, as we have pointed out, the pressor effect is not well shown even in so responsive an ainmal as the cat.

The Presence of Ergotoxine in Supposed Active Principles of a Non-Alkaloidal Nature

The feebly basic nature of ergotoxine, its acidic character, due to a phenolic hydroxyl, the colloidal condition of its salts in aqueous solution, its large molecular weight and its amorphous nature—all these properties may be held responsible for the fact that ergotoxine clings so tenaciously to the resins, fat, and colouring matters present in ergot, and have determined its presence in the various non-alkaloidal substances described as active principles by Kobert, Jacobj, and Meulenhoff. Of these Jacobj's chrysotoxin is the most clearly characterised ; on this account, and because it was the starting point of our own investigation, it will be convenient to deal first with the work of this observer.

(*a*) *Jacobj's Preparations*

Theoretically there is something to be said for the use of indifferent solvents of low boiling point, such as ether and light petroleum, and Jacobj (37) in so far achieved his object, that the decomposition of the active substance was probably less in his than, for instance, in Kobert's experiments. On the other hand, it is impossible to separate the active substance from inert constituents by the use of such solvents alone.

There are two ways of preparing the fat-free ethereal extract, which corresponds to Jacobj's crude chrysotoxin. In the first place the ergot can be extracted with ether ; the ether is then evaporated, and a large volume of light petroleum is added, which precipitates the 'chrysotoxin,' and leaves the oil in solution. Secondly, the ergot can be extracted first with light petroleum, which only removes the oil, and then with ether. Fatty oil is present in ergot to the extent of about 33 per cent., but the amount which is readily extracted by percolation with light petroleum amounted, in our experiments, only to about 25 per cent. of the weight of the ergot. Kraft also found that at least 5 per cent. of oil remained in ergot after the most careful extraction with light petroleum.

On this account we chose, as did Kraft, the first method, viz., extraction of the ergot with ether, and subsequent precipitation with light petroleum. We can confirm Kraft's statement that a thorough extraction with ether is difficult to achieve. 1 kilo. of ergot,

in No. 40 powder, was percolated with 10 to 12 litres of dry ether. Nearly all the oil was removed by the first few litres of the percolate. On evaporating this, finally in vacuo, there remained 350 c.c. of oil, which, when mixed with light petroleum (700 c.c.), gave a precipitate of a grey solid, weighing 0·36 gramme. This produced the vaso-motor reversal in a dose of 15 mgms. per kilo. of cat. From the solution of the oil in light petroleum 0·060 gramme of ergotinine was obtained by shaking with acids.

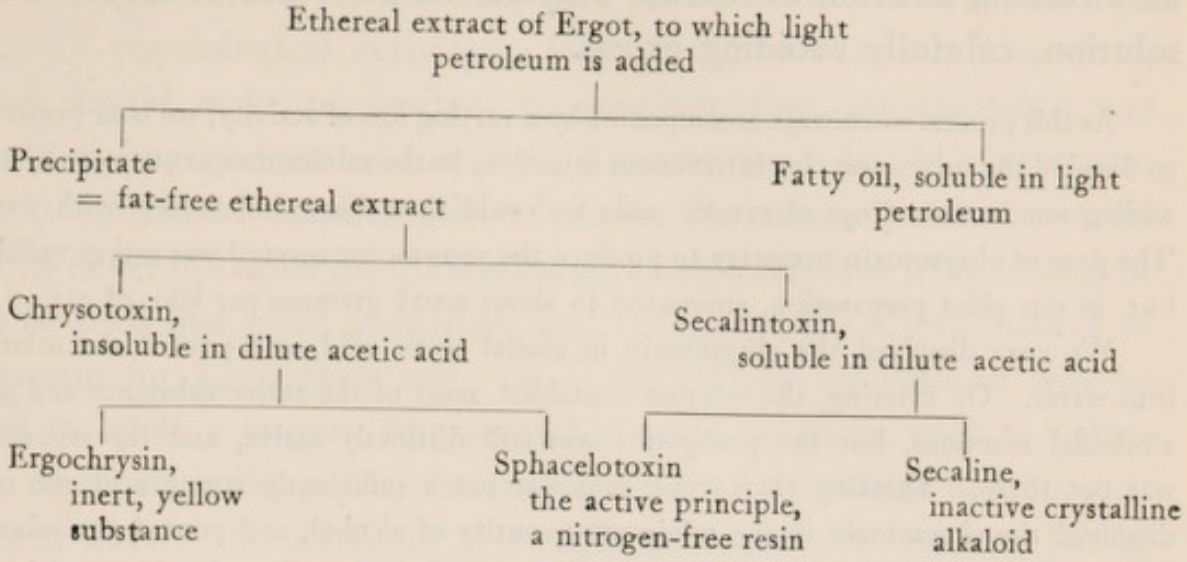
On continued percolation with ether, a yellowish-brown solid was obtained, which, after washing with light petroleum, weighed 0·156 gramme. The last three litres of the percolate together only left 0·013 gramme of a solid, which was very slightly soluble in ether. In all we had obtained 0·19 per cent. of the weight of the ergot as fat-free ether extract, but as the extraction proceeded so slowly and was incomplete, the experiment was abandoned.

Extraction with ether in a Soxhlet apparatus likewise gave bad results. 30 grammes of the same ergot powder were extracted for three weeks. The extract, after treatment with light petroleum, yielded ·07 gramme (0·23 per cent.) of a dark brown solid, which was tested physiologically. The vaso-motor reversal was nearly produced by a dose of 2·8 mgms. per kilo., and was complete after 5·6 mgms. We may put the dose at roughly 4 mgms. per kilo., corresponding to 1·6 grammes of ergot.

The ether-extracted powder in the Soxhlet apparatus was next exhausted for six days with 90 per cent. alcohol. A dose of the alcoholic extract corresponding to 4 grammes of ergot per kilo. of cat was necessary to produce the vaso-motor reversal. Assuming that all the active substance had now been extracted from the drug, we may conclude that the amount removed by ether in a Soxhlet apparatus was $\frac{4}{1\cdot6} = 2\frac{1}{2}$ times that which had been left in the ergot, so that after three weeks' extraction something like two-sevenths of the substance still remained behind. The only satisfactory method of ether-extraction seems to be the process employed by Kraft, based on Keller's assay method for ergot. Kraft shook the moistened ergot powder with ether for an hour at a time and repeated this ten times with fresh quantities of ether, thus obtaining 0·5 per cent. of fat-free extract and almost complete exhaustion (as compared with 0·19 and 0·23 per cent., which we obtained in a percolator and a Soxhlet apparatus respectively). In the light of these results we can readily understand Meulenhoff's conclusion, that simple percolation with ether removes very little of the active substance. He found that 2 kilos. of ergot, after percolation with 5 kilos. of ether, lost less than 20 per cent. of the active substance, as measured by toxicity to cocks. We also examined the solvent action of acetone on ergot. It resembles that of ether more than that of alcohol. By percolation with acetone and subsequent treatment of the extract with light petroleum we readily got 0·3 per cent. of a fat-free extract, closely resembling the extract obtained more slowly with ether.

According to Jacobj (37) the fat-free ether extract contains non-basic chrysotoxin and alkaloidal secalintoxin, both compounds of the active principle sphacelotoxin with the inert substances, ergochrysin

and secaline respectively. His conception of the relationship of these substances will be clear from the following diagram :—



Ergochrysin sphacelotoxin, and, therefore, also chrysotoxin, are, according to Jacobj, non-nitrogenous. Chrysotoxin in doses of 0·1 gramme produced the characteristic violet coloration of the cock's comb. The alkaloid secalintoxin had the same effect, but was about five times as potent. By repeated extraction with acetic acid chrysotoxin was changed to ergochrysin, which had the same chemical properties, but was physiologically inert. Hence, according to Jacobj, chrysotoxin must be a *compound* of ergochrysin and the active principle sphacelotoxin. Secalintoxin was obtained from crude chrysotoxin by extraction with acid, and was itself separated into the crystalline alkaloid secaline and the non-nitrogenous resin sphacelotoxin by mere treatment with neutral organic solvents. It hardly needs pointing out that neither chrysotoxin nor secalintoxin had any real claim to be considered a chemical compound. We have already given reasons for identifying secaline with Tanret's ergotinine ; ergochrysin being admittedly inactive, does not directly concern us : the important point is the nature of sphacelotoxin.

Using the vaso-motor reversal as a measure of activity, we, therefore, repeated Jacobj's experiments.

A quantity of crude 'chrysotoxin' was purified by fractional precipitation of its solution in ether by light petroleum. It was a

yellow powder very soluble in ether. Several specimens were converted into the water-soluble sodium-compound, by the addition of an alcoholic solution of sodium ethylate to the ethereal chrysotoxin-solution, carefully avoiding excess.

As this process was always accompanied by a varying loss of activity, we later preferred to dissolve the substances, for intravenous injection, in the minimum quantity of alcohol; adding one or two drops of caustic soda we could then dilute indefinitely with water. The dose of chrysotoxin necessary to produce the vaso-motor reversal was rather variable, but, in our chief preparation, amounted to about 0·025 gramme per kilo. of cat.

We next dissolved the chrysotoxin in glacial acetic acid, and poured the solution into water. On filtering, the solution contained most of the active substance and gave alkaloidal reactions, but the precipitate was still distinctly active, and the separation was not sharp. Thinking that acetic acid was not a sufficiently strong acid, we next dissolved the chrysotoxin in the minimum quantity of alcohol, and poured the solution into 5 per cent. aqueous sulphuric acid.[1] The precipitate was collected and treated in the same way. After this process had been performed three times the activity of the precipitate had fallen to about one-seventh of its original value, since 0·170 gramme per kilo. was now required to produce the vaso-motor reversal in a cat, instead of 0·025 of the original preparation. Continued treatment with sulphuric acid still further reduced the activity of the precipitate.

All the acid filtrates were mixed and made alkaline with sodium carbonate. In this way a white flocculent precipitate of alkaloids was obtained, soluble in caustic soda and constituting about 1 per cent. of the chrysotoxin employed. The alkaloidal precipitate was found to contain a considerable part of the activity of the original chrysotoxin, a dose corresponding to 0·07 gramme of chrysotoxin per kilo. producing the vaso-motor reversal. The mixture of alkaloids thus obtained obviously corresponded to Jacobj's 'secalintoxin.' When its ethereal solution was evaporated it deposited crystals of ergotinine (= secaline), which were identified by the melting point (219°). The mother liquor left behind an amorphous alkaloid closely resembling the pure alkaloid subsequently named ergotoxine, and at the same time corresponding to Jacobj's 'sphacelotoxin.' The same separation of the alkaloidal constituents from chrysotoxin was per-

1. Kraft (46) has since shown that the sulphate of ergotoxine (hydroergotinin) is particularly insoluble, a fact which, no doubt, in part accounts for our comparative failure to free the chrysotoxin from active alkaloid.

formed with great care by Kraft, using glacial acetic acid. After four successive precipitations with water, he found that the precipitate still contained traces of alkaloid, just as we found that it retained traces of physiological activity. The only consideration, therefore, which should make us hesitate to conclude that sphacelotoxin was merely an impure form of our alkaloid ergotoxine is Jacobj's statement that it contained no nitrogen. It must be remembered, however, that he never obtained his sphacelotoxin completely free from nitrogen, that he used but small quantities, and that he used sodium instead of potassium for Lassaigne's test. It is difficult to undertsand why he failed to apply a reagent immeasurably more sensitive, such as potassium mercuric iodide, which detects one part of ergotoxine in 2,000,000 parts of water. Similarly, by the use of alkaloidal precipitants, Jacobj might have detected the presence of alkaloids in active specimens of chrysotoxin. In any case, his failure to detect nitrogen qualitatively can be of no significance when we consider his quantitative results. Two determinations gave him for the mixture of alkaloids, called secalintoxin, 11·71 and 11·79 per cent. of nitrogen. In the one constituent, secaline, he found 11·62 per cent.; obviously, therefore, the other constituent, sphacelotoxin, must have had about the same nitrogen content. Compare with this the nitrogen contents of ergotinine, 11·5 per cent., and of ergotoxine, 11·2 per cent. (Barger and Carr). Similarly the carbon content of secalintoxin was but slightly below that of ergotinine and of ergotoxine. Apart from the supposed absence of nitrogen, sphacelotoxin corresponds in all characters, chemical and physiological, to an impure and partially decomposed mixture containing probably up to 50 per cent. of ergotoxine. Jacobj records a marked effect on the cock with 5 and with 8 mgms. sphacelotoxin; we obtained a very marked and typical effect with 2 mgms. of ergotoxine (see p. 266). The great solubility in alcohol and the green colour developed on standing are both characteristic of ergotoxine, while the yellow colour of fresh preparations is due to a tenaciously adhering colouring matter. Jacobj's partial success in separating sphacelotoxin from secaline as a lead salt corresponds to the separation of ergotoxine from ergotinine by dilute caustic soda (Barger and Carr).

We conclude, therefore, that the only active substance present in Jacobj's preparations was the alkaloid ergotoxine; that this, in relatively pure form, constituted his sphacelotoxin; that, mixed with ergotinine, it was present in secalintoxin; and that it occurred as a contamination to the extent of about 2 per cent. in chrysotoxin.

(*b*) *The Sphacelinic Acid of Kobert*

Jacobj regarded chrysotoxin as the sphacelinic acid of Kobert (20) in a pure form. He found that a specimen of this substance, prepared according to the method of Kobert and Bombelon, consisted for two-thirds of an inert fatty substance soluble in light petroleum, and, for the rest, of an active substance, which resembled chrysotoxin that had been decomposed by alkali.

Our view that Jacobj's chrysotoxin owed its activity to contamination with an active alkaloid (ergotoxine) applies equally to Kobert's sphacelinic acid. We put this view to the test of experiment, and prepared sphacelinic acid according to Kobert's original method.

Ergot powder was percolated successively with light petroleum, ether, and 90 per cent. alcohol. The residue left on evaporation of the alcoholic percolate was extracted first with water, and then with phosphoric acid. The extraction with phosphoric acid at this stage constitutes a slight departure from Kobert's method, and replaces his preliminary extraction of the ergot with 3 per cent. hydrochloric acid. As the chlorides of the ergot alkaloids are very difficultly soluble, we considered that our variation of Kobert's method would be more effective in removing the alkaloids. The brown resin which remained behind after extraction with phosphoric acid was finally dissolved in caustic soda, and precipitated by phosphoric acid. In this way we obtained from 500 grammes of ergot 0·6 gramme of an almost fat-free greyish-brown powder, showing in a high degree the physiological activity characteristic of ergotoxine. A dose of 3 mgms. per kilo. was more than sufficient to produce a complete vaso-motor reversal in the cat.

The preparation was about as active in this respect as the most active chrysotoxin which we had prepared.

This statement scarcely agrees with Jacobj's view of chrysotoxin as a purer form of sphacelinic acid—at least, if by 'purer' we mean richer in activity. On the other hand, chrysotoxin contains the inert yellow colouring-matter in a purer form than sphacelinic acid. Compared with the sphacelinic acid used by Grünfeld and by Jacobj

(the latter's specimen was two-thirds fat) our preparation was presumably much more active.

In order to prove that our sphacelinic acid owed its activity to an alkaloidal contamination, a solution of 0·5 gramme in dilute caustic soda was poured into 100 c.c. of 10 per cent. phosphoric acid. The precipitate was filtered off at the pump, and washed with water; it was then redissolved in a little caustic soda, and again poured into acid. This process was gone through three times. The filtrates, which were quite clear, gave alkaloidal reactions, but not very strongly. By making alkaline with sodium carbonate and shaking out with chloroform we collected the minute quantity of alkaloid, and injected a dose equivalent to 10 mgms. of the original sphacelinic acid into a cat of 3 kilos. A very distinct ergotoxine effect was produced, but the vaso-motor reversal was incomplete. We then gave a corresponding dose of the sphacelinic acid which had been extracted with phosphoric acid. It also showed activity, though it was much less active than the original preparation.

At first we were at a loss to explain the fact that by methods calculated, as we believed, to remove the whole of the alkaloid, we could not remove the whole of the ergotoxine-activity, but a study of the properties of pure ergotoxine salts afforded us an explanation. The phosphate of ergotoxine, for instance, can form a typically colloidal solution in water, containing as much as 1 per cent. Like many other colloids it is, however, precipitated by any well-ionized electrolyte; therefore by all salts and strong mineral acids, but not by the weaker acids. Phosphoric acid alone does not precipitate the ergotoxine phosphate, so that the alkaloid might have been extracted, but the sodium phosphate formed inevitably precipitated much of the ergotoxine phosphate on to the sphacelinic acid. In the presence of excess of sodium phosphate we have found the true solubility of ergotoxine phosphate to be of the order of 1 : 20,000. The three filtrates of rather more than 100 c.c. each might contain very roughly 10 mgms. of ergotoxine, and the dose administered would be $\frac{1}{50}$th of this, or something like $\frac{1}{5}$th mgm. For complete vaso-motor reversal (in the cat of 3 kilos.) about 1·5 mgm. of ergotoxine phosphate would be required.

In another somewhat similar experiment we were more successful in effecting a complete separation.

An alcoholic extract was freed from fat by means of light petroleum, and was then suspended in water. 50 c.c. of the mixture, corresponding to 100 grammes of ergot, were poured into 500 c.c. of 5 per cent. hydrochloric acid. The liquid was then filtered and the process repeated five times. The last precipitate was dissolved by means of a little caustic soda, and a dose equivalent to 10 grammes of ergot was injected intravenously into a cat. The only noticeable effect was a temporary depression of the heart's action. A dose of the alkaloid extracted from the six filtrates and also equivalent to 10 grammes of ergot was next given to the same cat, and produced a marked rise of blood-pressure, with subsequent complete vaso-motor reversal, so that even 1 mgm. of the suprarenal active principle produced a fall of blood-pressure.

In this way it was proved that the whole of the active principle producing the vaso-motor effects here in question has basic properties and can be removed by acids. Very instructive in this connection are experiments made by Grünfeld (30) under Kobert's direction with a preparation which he calls ' crude sphacelinic acid ' (Rohsphacelinsäure). This substance, Grünfeld explains, was obtained in the purification of ergotinine of Tanret. The crude ergotinine was dissolved in chloroform and ether added until the portion remaining behind in the ether-chloroform solution was colourless. The resinous precipitate, produced by the addition of ether, was called sphacelinic acid, because of its action on the cock's comb, and apparently not for any chemical reason. Chemically speaking, it cannot be doubted that the precipitate contained a considerable quantity of ergotoxine, which, as has been shown, is but slightly soluble in ether. Grünfeld found that doses of 1, 1 and 0·6 gramme of the precipitate gave what he describes as ' a most characteristic picture of gangrenous ergot poisoning,' and adds that the effect was equivalent to that of 7·0 grammes of ' pure sphacelinic acid, which, moreover, was administered only six months after the harvest.'

This experiment from Kobert's own laboratory points to the conclusion that the true gangrenous effects on the cock's comb, like the vaso-motor effects on the cat in our own experiments, are produced by an alkaloidal and not by an acid constituent of ergot. In the light of our later experiments, described in a former section of this paper, it can now be definitely stated that this basic principle is the alkaloid ergotoxine, which in the pure state not only produces the

vaso-motor reversal in the cat, but also the gangrene of the cock's comb and other effects regarded by Kobert as characteristic of sphacelinic acid. The chain of evidence, therefore, seems complete for the conclusion that sphacelinic acid, like chrysotoxin, owes its activity to adherent ergotoxine.

The Preparations of the British Pharmacopoeia

From the physical and chemical properties of ergotoxine already described, it will be clear that its occurrence in any considerable proportion in the official aqueous extract (Extractum Ergotae Liquidum) is not probable. Small traces of an alkaloid, soluble in chloroform, and giving the physiological reactions of ergotoxine, can be obtained from most specimens of the extract. Since ergot always contains a considerable quantity of di-acid potassium phosphate, these traces of ergotoxine are probably dissolved as the phosphate, which, in the presence of salts, is very slightly but distinctly soluble. In our experience, however, the stimulant effect of the liquid extract on involuntary muscle, as indicated by its power of raising the blood-pressure, is much too great to be accounted for by the amount of ergotoxine present, as indicated by the vaso-motor reversal, and by the amount which can be extracted by chloroform. The same discrepancy appears to have been met with by Cushny (50) in his experiments on the effect of ergot extracts on the uterus. With regard to the pressor effect, Dixon (42) has pointed out that liquid extracts have a marked augmentor effect on the action of the heart, and the disproportion between the rise of blood-pressure and the amount of ergotoxine present might, in part, be accounted for by the presence of some cardiac stimulant principle. According to Plumier (40), the fluid extract which he examined contained a principle which caused rise of both aortic and pulmonary pressure, due to constriction of both systemic and pulmonary arterioles, the effect of 'ergotinine' being smaller, and confined to the systemic vessels. Recently Meltzer and Auer (48) have described an effect of the fluid extract (U.S.P.) on the movements of the stomach and intestines.

They regard the augmentation of movements and increased sensitiveness to vagus-stimulation which they observed as similar to the effects obtained by one of us (43) with chrysotoxin and other ergotoxine-containing preparations. We have no indication as to the richness in ergotoxine of the particular fluid extract which they used, but, from general experience of such preparations, we are disposed to regard it as doubtful whether the phenomena which they observed are due to ergotoxine at all. Especially significant is their insistence on the striking increase of gastro-intestinal movement, following injections of the extract. We can only confirm the statement in the former paper, that the effect, in this direction, of ergotoxine, and of preparations owing their activity entirely to it, is comparatively slight and inconstant. The effect on intestinal movements of a complex fluid such as the liquid extract, containing, apart from principles the action of which is peculiar to ergot, choline and various other vascular depressants (ergotinic acid, etc.), seems to us to need a more critical analysis before any great importance is attached to it as a specific action.

The same criticism applies more obviously to the numerous descriptions of a fall of blood-pressure as the characteristic effect of injecting ergot preparations. (Cf. Sollmann and Brown (41), who give references to other similar papers.)

In regard to the amount of ergotoxine present, the position of the Extractum Ergotae (' Ergotin ') of the British Pharmacopoeia is not widely different from that of the liquid extract. Although the ergot is, in the first place, extracted with 60 per cent. alcohol, nearly the whole of the alkaloid so extracted is subsequently removed in the resin which is precipitated by the addition of hydrochloric acid after removal of the alcohol. It may, indeed, be said that the ergotoxine-content of ' ergotin ' is in inverse relation to the care with which the official instructions are carried out. Like the liquid extract, carefully made specimens of the official ergotin appear to have a more marked pressor effect than is accounted for by the small amount of ergotoxine present, though the question of the existence of a second stimulant principle must in our opinion be regarded as not finally decided until it can be separated from the physiologically active substances.

Of the remaining pharmacopoeial preparations, the infusion does not require consideration apart from the liquid extract, and the *injectio hypodermica* is merely a solution of ergotin. The ammoniated tincture contains, as might be expected, a larger proportion of ergotoxine than any other official preparation, as indicated by the physiological test. It does not, however, contain by any means the whole of the ergotoxine of the ergot from which it is prepared. It is doubtful whether there is any advantage in the use of ammonia in the extraction, and its presence in the tincture may facilitate the decomposition of the ergotoxine.

Vahlen's Clavin

In considering the possibility that pharmacopoeial preparations, such as the liquid extract, may contain an active principle distinct from ergotoxine, Vahlen's recent claim (44) to have isolated a water-soluble active principle is of interest. Starting from the discrepancy between the extensive clinical use of the liquid extract and the fact that all the 'active principles' hitherto prepared by pharmacologists were insoluble in water, Vahlen succeeded in obtaining from watery extracts a crystalline neutral substance. He stated that the substance clavin produced, in the pregnant animal, a co-ordinated peristaltic activity of the uterus of the type seen in normal labour, but that it was devoid of any toxic properties, and was thereby sharply differentiated from other active principles of ergot.

As soon as clavin was commercially obtainable we submitted a sample to physiological experiment. The animals used were cats, pregnant and non-pregnant, and rabbits, of which one was in the latest stage of pregnancy. The conditions observed by Vahlen himself have in several instances been reproduced, the animal being immersed in a saline bath at 37°, and anaesthesia being produced by urethane. In all, three commercial specimens, obtained at different times, were used, and, in addition, a specimen prepared in the laboratory by ourselves. In no case was any trace observed of the action described by Vahlen; a temporary slowing of the respiration and, in one case, a slight and evanescent depression of the heart's action

were the only effects observed. This last effect was accounted for by the presence of acid potassium phosphate, with which this specimen was contaminated. A similar absence of effect of clavin on the uterus has been observed by Cushny (50).

The following is the complete record of one of our experiments, made on a cat in advanced pregnancy :—

CAT, weight 3 kilos. Anaesthetised by injection of 4·5 grammes of urethane. The blood-pressure was recorded from the carotid artery, and injections made into the external jugular vein. The abdominal wall was not opened until the abdomen had been submerged in a bath of physiological saline at 37°.

11.50 a.m. Abdominal wall opened in the bath of warm saline. Uterus, containing two foetal sacs in each horn, was flaccid and inactive. It remained so till

11.55 a.m., when it contracted for twenty to twenty-five seconds, and then relaxed again. The contraction affected both horns equally, and appeared uniform in distribution.

11.59 a.m. A similar contraction, again lasting twenty to twenty-five seconds.

12.2 p.m. Contraction for thirty seconds.

12.6 p.m. Contraction for thirty to forty-five seconds.

12.7 to 12.9 p.m. Injection of 70 mgms. of clavin, dissolved in 7 c.c. of physiological saline, into the jugular vein.

12.10 p.m. Contraction of the uterus, lasting rather more than thirty seconds.

12.14¼ p.m. Contraction for rather more than one minute.

12.17 p.m. Contraction for about thirty seconds.

12.18 p.m. Contraction for about ten seconds.

12.19 to 12.20 p.m. Ether given by inhalation.

12.22 p.m. Contraction of uterus for twenty seconds.

12.22½ p.m. Contraction for fifteen seconds.

12.23 p.m. Ether for one minute.

12.24 p.m. Contraction for twenty seconds.

12.25 p.m. Contraction for fifteen seconds.

12.25½ p.m. Contraction for twenty seconds.

12.27 p.m. Contraction for twenty seconds.

12.29 p.m. Contraction for twenty seconds.

Beside these larger contractions, small irregular waves of contraction have all along been seen passing over the uterus. The main contractions have been, since the injection of clavin, distinctly less powerful than before, though rather more frequent. A second injection of 80 mgms. of clavin was given at 12.38 p.m., and the uterus watched till 12.59 p.m., with the result that no alteration in its activity was detected. The same normal, rhythmic contraction of the organ proceeded at intervals of one to three minutes, each lasting for twenty to thirty seconds.

1.0 p.m. Injection of 2 mgms. of ergotoxine phosphate, in 1 c.c. of distilled water, into the jugular vein. Breathing becomes feeble and blood-pressure tends to fall as respiration stops. Artificial respiration given.

1.1 to 1.2 p.m. Contractions of the normal type occurred.

1.3 p.m. Strong contraction, very marked between the foetal sacs.

1.5 p.m. Uterus still contracting, strongly between the foetal sacs.

1.7½ p.m. Strong rings of contraction, originating between and passing over the sacs towards the body of the uterus. The whole organ is tense and pale.

1.12 p.m. The waves of contraction continue. The tone of the whole organ seems slightly less.

1.15 p.m. Contraction continues.

1.15½ p.m. Injection of 0·1 mgm. of the suprarenal active principle intravenously.

1.16 p.m. Marked relaxation of the uterus, the outlines of the foetal sacs, which had been sharply defined by the constrictions between them, becoming far less apparent.

1.17 p.m. Reappearance of the tonic contraction.

1.18 p.m. Peristalsis recommencing.

1.35 p.m. Fairly vigorous peristalsis still continues. Cat killed.

In this experiment, therefore, 150 mgms. of clavin failed to produce any significant effect on the uterus, the slight weakening and acceleration of the normal rhythm being possibly due to the exposure in the saline bath, and, in any case, of no importance. Subsequently 2 mgms. of ergotoxine produced tone and marked peristaltic activity in the uterus, which, at that time, had already been exposed to the saline for over one hour.

Fig. 8 shows the effect of clavin and ergotoxine on the graphically recorded contractions of the uterus of another cat in early pregnancy.

Having failed to produce any physiological effect with clavin, and having satisfied ourselves that our specimens corresponded chemically to Vahlen's description, we proceeded to a more thorough investigation of its nature, and prepared a larger quantity by a slight modification of Vahlen's method.

22 kilos. of ergot were extracted with water; the extract was concentrated to 22 litres, and precipitated with 1500 grammes of barium hydrate; the precipitate was washed with 13 litres of water. On removal of the excess of baryta from the filtrate and washings, by means of sulphuric acid (about 50 grammes of barium sulphate being formed), the solution was concentrated *in vacuo* to a small bulk, mixed with 10 kilos. of silver sand, and dried completely *in vacuo* at 100°. The resulting product was extracted three times with 92 per cent. alcohol, and the resulting alcoholic solution (about 30 litres)

was evaporated. The dark brown residue was then dissolved in the minimum quantity of boiling 75 per cent. alcohol (3 litres); on standing a crystalline deposit was formed, which was recrystallised twice from 75 per cent. alcohol. The yield was 15 grammes, or about 0·07 per cent. of the ergot employed.

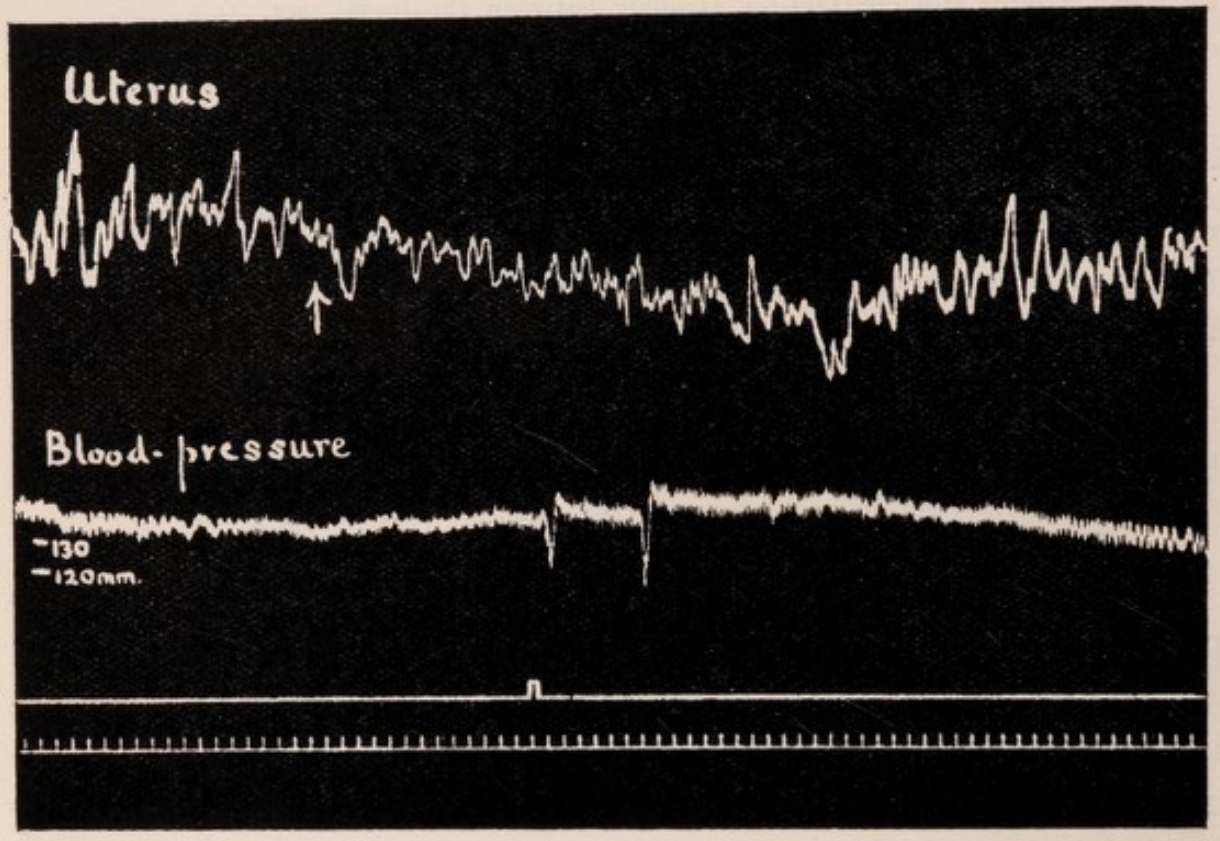


FIG. 8A

Cat, 3 kilos. Early pregnancy. Urethane. Abdomen opened in saline bath at 37°. Record of uterine contractions and carotid blood-pressure. Contraction of the uterus pulls down the lever, the movements being magnified twofold.

Effects of injecting 100 mgms. of clavin, dissolved in water, intravenously. No perceptible alteration in uterine tone or rhythm. The arrow indicates the time of injection for the tracing from the uterus.

For analysis the substance was recrystallised once with animal charcoal, and finally once without animal charcoal. It melted in a sealed capillary tube at 265°. Heated in an open tube, it yielded a crystalline sublimate, but also underwent a slight decomposition, and gave an odour very similar to that which leucin gives when heated in the same manner. The substance consisted of minute feathery needles, but sometimes rhomb-shaped plates were observed. With copper acetate it yielded a blue copper salt, slightly soluble in water. The general behaviour of the substance left no doubt as

to its identity with clavin. This identity was further supported by analyses, which, at the same time however, showed that the substance was not a chemical individual.

I. 0·1381 gramme gave 0·2630 gramme CO_2 and 0·1168 gramme H_2O; C = 51·94 per cent., H = 9·40 per cent.

II. 0·1607 gramme gave 0·3072 gramme CO_2 and 0·1350 gramme H_2O; C = 52·14 per cent., H = 9·33 per cent.

III. 0·1792 gramme gave 15·7 c.c. N at 7° and 770 mm.; N = 10·8 per cent.

IV. 0·1593 gramme gave 14·4 c.c. N at 10° and 765 mm.; N = 10·9 per cent.

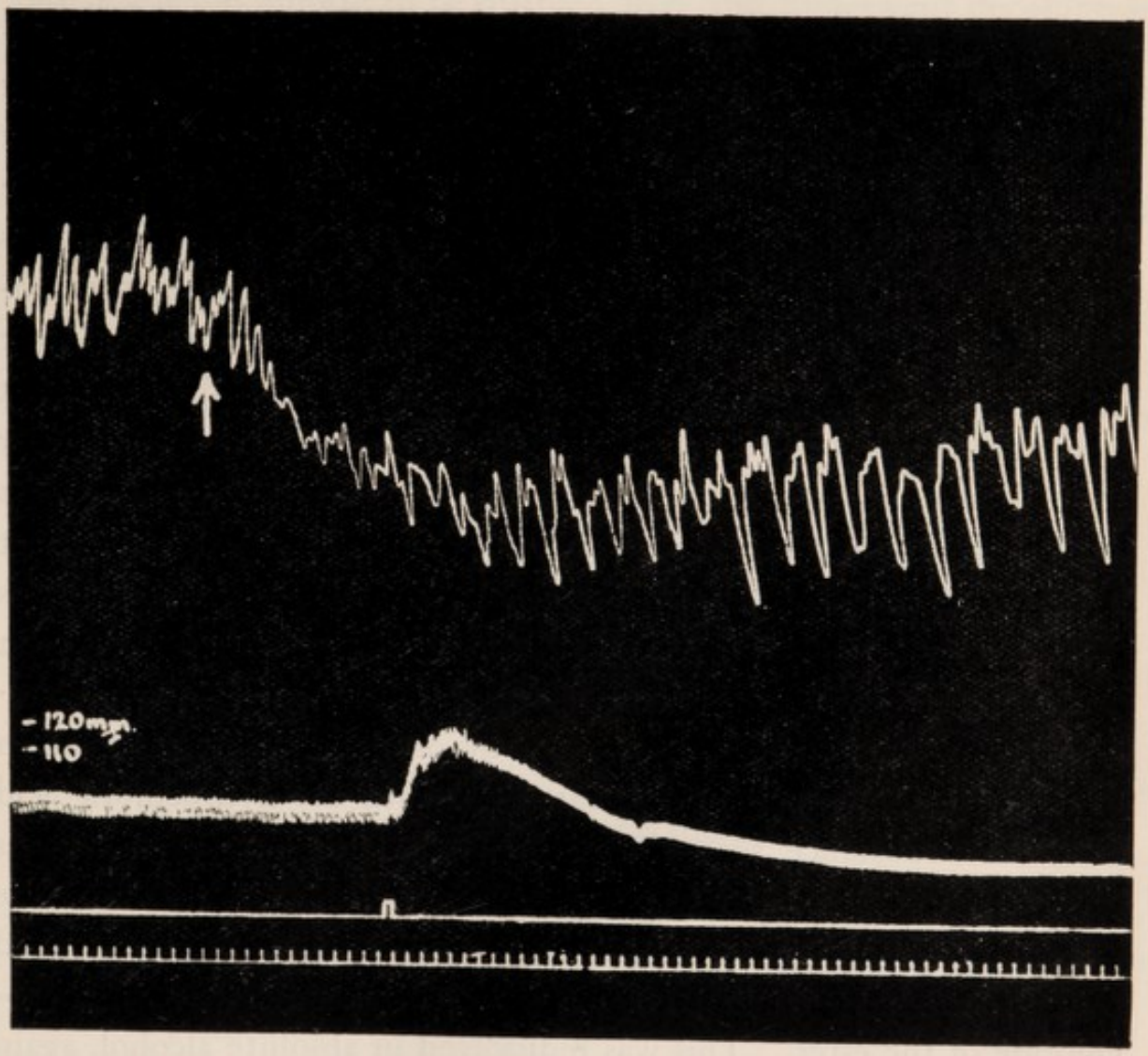


FIG. 8B

Same animal as in FIG. 8A

Effect of a later injection of 2 mgms. ergotoxine phosphate dissolved in water. The condition of the animal being poor, through long exposure of the viscera to saline, there is no great rise of blood-pressure, and only a moderate effect on the uterus. The tone and rhythm are, however, distinctly augmented.

Molecular weight determinations gave the following results :—

I. 0·215 gramme in 14·46 grammes water gave $\Delta = 0·24°$; M.W. = 117.

II. 0·100 gramme in 14·76 grammes glacial acetic acid gave $\Delta = 0·030°$; M.W.= 876. 0·230 gramme in 14·76 grammes glacial acetic acid gave $\Delta = 0·093$; M.W. = 650.

III. By a vapour pressure method due to one of us,[1] in aqueous solution, 0·1049 gramme in 3·9877 grammes water was intermediate between urea solutions of 0·18 and 0·22 gramme molecules per litre ; M.W. 118 to 146, mean 132.

IV. By the same method in glacial acetic acid solution 0·138 gramme in 5 grammes of glacial acetic acid was intermediate between benzil solutions of 0·14 and 0·18 gramme molecules per litre ; M.W. 150 to 194, mean 172.

As the analytical results were not in agreement with a single formula of the magnitude indicated by the molecular weight determinations, the substance was converted into its copper salt, by boiling with saturated copper acetate solution. As did Vahlen, we succeeded in separating the copper compound into two fractions differing as regards their solubility in water. Regenerating the substance from the less soluble copper salt, which was by far the larger fraction, we found it to have a composition but slightly different from that of the original mixture (C = 52·6 per cent., H = 9·7 per cent.). Since the properties of the substance suggested that it was leucin in an impure form, and since, as is well known, it is very difficult to purify amino-acids by recrystallisation, we adopted Fischer's process of separation by distillation of the esters.[2] The presence of leucin in ergot was suspected long ago by Buchheim (10).

5·5 grammes of clavin, prepared by ourselves, was suspended in 50 c.c. of absolute alcohol, and saturated with dry hydrochloric acid gas. The substance soon dissolved completely ; the solution was then evaporated *in vacuo* at a temperature below 60° to remove water formed during the reaction ; a light brown syrup remained behind, which on cooling formed a mass of crystals of the hydrochloride of the ester. This was redissolved in absolute alcohol, again saturated, treated with potassium carbonate and caustic soda, and extracted with ether in the manner described by Fischer. During

1. *Journ. Chem. Soc.*, LXXXV, p. 286, 1904.
2. *Ber. deutsch. chem. Gesellsch.*, XXXIV, p. 433, 1901.

this process a small quantity of an amino-acid was apparently reformed and became suspended in the ether in fine crystals, which were filtered off.

On fractionating the mixture of esters at 9 mm. distillation began at 79°, and 3·4 c.c. distilled over below 85°.

The thermometer then rose rapidly, remaining for a short time at 126° to 127° ; a few drops of a somewhat viscid and slightly coloured distillate were collected between 120° and 130°. On further heating, the residue in the flask apparently boiled at a still higher temperature, but it was too minute to be distilled over and underwent decomposition. The boiling-point of the lower fraction corresponded very closely to that given by Fischer for leucin ester. The boiling-point of the higher fraction in conjunction with its solubility in water (which excludes phenyl-alanin) indicated that it consisted chiefly of aspartic ester, possibly with traces of glutaminic ester.

For identification the leucin fraction was hydrolysed by boiling with twenty times its volume of water for five hours ; plates were obtained apparently identical in all respects with a specimen of leucin prepared from casein.

On analysis—

0·1239 gramme gave 0·2494 gramme CO_2 and 0·1110 gramme H_2O. Found: C = 54·68 per cent., H = 9·95 per cent. Calculated for $C_6H_{13}O_2N$: C = 54·96 per cent., H = 9·92 per cent.

The copper salt was wholly insoluble in methyl alcohol, thus indicating the absence of iso-leucin.[1] A measurement of the optical rotation in 20 per cent. hydrochloric acid gave the following results :—

$$a_D = +0{\cdot}79^\circ; \quad c = 5\%; \quad l = 1 \text{ dm}; \quad [a]_D = +15{\cdot}8^\circ$$

The value given by Schulze for *l*-leucin under these conditions is

$$[a]_D = +17{\cdot}5^\circ.$$

The amount of the fraction collected between 120° and 130° was too small to admit of an attempt at hydrolysis, so the impure ester was analysed as such.

1. Ehrlich, *Ber. deutsch. chem. Gesellsch.* XXXVII, p. 1809, 1904.

0·1125 gramme gave 0·2060 gramme CO_2 and 0·0870 gramme H_2O. C = 49·9 per cent., H = 8·6 per cent.; calculated for aspartic ester, $C_8H_{15}O_4N$: C = 50·8 per cent., H = 8·0 per cent.

We thus proved that the specimen of clavin prepared by ourselves consisted almost completely of leucin and aspartic acid. The analytical results of the original mixture, given above, indicate that its approximate composition was six parts of leucin to one part of aspartic acid. Such a mixture would have C = 52·3 per cent., H = 9·3 per cent., N = 10·7 per cent.; the mean of the values found was: C = 52·1 per cent., H = 9·4 per cent., N = 10·8 per cent.

We next examined two commercial specimens of clavin by the same method. The first of these contained 40 per cent. of di-hydrogen potassium phosphate. On esterification 1·93 grammes left undissolved 0·4 gramme of potassium chloride (equivalent to 0·73 gramme or 38 per cent. of di-hydrogen potassium phosphate). On distillation the same fractions were obtained in the same relative proportions as in the case of the specimen prepared by ourselves.

The leucin, obtained by hydrolysis, was analysed:—

0·1231 gramme gave 0·2468 CO_2 and 0·1061 gramme H_2O. C = 54·68 per cent; H = 9·58 per cent. $C_6H_{13}O_2N$ requires · C = 54·96 per cent.; H = 9·92 per cent.

The second commercial specimen yielded only 1 per cent. of ash. 4 grammes gave 3·2 grammes of leucin ester and 0·3 gramme of a higher boiling fraction. This specimen contained 52·6 per cent. of carbon and 10·3 per cent. of hydrogen.

The values given above for the molecular weight of 'clavin' in aqueous solution (117 and 132; Vahlen found on the average 122) are in agreement with those of leucin (131) and aspartic acid (133). In acetic acid solution, however, we obtained a higher value (172), and the mean of Vahlen's determinations in this solvent was 247·5. It was on this higher value that he founded his formula $C_{11}H_{22}O_4N_2$. We have found, however, that in glacial acetic acid solution the molecular weight of pure leucin (from casein) is abnormal.

0·084 gramme in 14·74 grammes acetic acid gave $\Delta = 0·044°$; M.W. = 506.

0·210 gramme in 14·74 grammes acetic acid gave $\Delta = 0·168°$; M.W. = 332.

No significance can, therefore, be attached to the molecular weight determinations of 'clavin' in acetic acid.

The other properties of 'clavin,' described by Vahlen, are consistent with the fact that it is chiefly composed of leucin and aspartic acid. The separation into two copper salts a more soluble (that of aspartic acid) and a less soluble (that of leucin) is thus rendered intelligible. The percentage of chlorine in the hydrochloride of leucin is 21·2 per cent., and in that of aspartic acid 20·0 per cent.; Vahlen found for the mixture 22·1 per cent.

The result of our chemical investigation of clavin completely explains our failure, and that of Cushny, to obtain any effect on the uterus with this substance.

Summary and Conclusion

1. Of the physiological effects described as characteristic of ergot, the alkaloid ergotoxine produces in very small dosage :—

(*a*) The effects ascribed by Kobert to sphacelinic acid, and by Jacobj to sphacelotoxin, viz. :—Ataxia, dyspnoea, salivation, gastro-intestinal irritation, and gangrene.

(*b*) The stimulant effect on plain-muscular organs—in particular the arteries and the uterus—and the subsequent selective sympathetic motor paralysis given by many ergot preparations (Dale).

2. Ergotoxine, $C_{35}H_{41}O_6N_5$, is the hydrate of the crystalline alkaloid ergotinine (Tanret), $C_{35}H_{39}O_5N_5$. Ergotoxine is itself amorphous, but yields crystalline salts, and further differs from ergotinine in being very soluble in alcohol. Its most important difference, however, is its intense physiological activity, ergotinine being but slightly, if at all, active when pure. Either alkaloid can readily be transformed into the other by chemical means.

3. The action of the pharmacopoeial extracts appears too great to be accounted for by the small amount of ergotoxine which they contain, and it seems likely that some other active principle is present in them.

4. This other hypothetical principle is not the 'clavin' of Vahlen, which is a mixture of leucin and aspartic acid, and is pharmacologically inert.

It will, perhaps, be useful to express in tabular form our conception of the relation to the ergot alkaloids, ergotinine and its hydrate ergotoxine, of the various other 'active principles' obtained from ergot, based on our own experiments and on those of other observers.

Ecboline and ergotine (Wenzell) = Mixtures of alkaloids, containing choline (Meulenhoff).

Amorphous ergotinine (Tanret) = Impure mixture of ergotinine and ergotoxine.

Picrosclerotine (Dragendorff) = Ergotinine, possibly mixed with ergotoxine.

Sclerocrystalline (Podwyssotski) = Ergotinine.

Sphacelinic Acid (Kobert) = Inactive resin with adherent alkaloid.

Cornutine (Kobert) = An alkaloidal resin, probably containing some ergotoxine, and also some other active substance, which may be a decomposition product of ergotoxine.

Cornutine (Keller) = Impure mixture of ergotinine with ergotoxine.

Chrysotoxin (Jacobj) = Inactive yellow colouring matter with a small proportion of adherent alkaloid.

Secalintoxin (Jacobj) = Mixture of ergotinine and ergotoxine.

Sphacelotoxin (Jacobj) = Impure ergotoxine.

Hydroergotinin (Kraft) = Synonym for ergotoxine.

It will be clear from the above table that Tanret's conception of the nature of the active constituent of ergot approximated much more closely to that held by ourselves and by Kraft than did those of the intervening observers. The only modification necessary of the view put forward by Tanret, albeit an important one, was the recognition that his so-called 'amorphous ergotinine' was not merely physically different from the crystalline, but was largely composed of a separate though closely-related chemical individual, and that to this amorphous alkaloid, and not to its crystalline anhydride, were due the more prominent physiological effects of ergot. Led astray by Tanret's identification of the two alkaloids, and finding that the crystalline, and, therefore, pure, ergotinine was almost inert, other observers such as Kobert and Jacobj were induced to search in other

directions for a principle which Tanret had already so nearly isolated. Now that the nature of this principle is clear, a considerable simplification both of nomenclature and conception should be possible in the hitherto bewildering pharmacology of ergot.

We have pleasure in expressing our indebtedness to Messrs. F. H. Carr and W. C. Reynolds, whose assistance in preparing extracts on a large scale alone made our work possible; also to Mr. A. J. Ewins for valuable assistance in the details of the chemical investigation.

BIBLIOGRAPHY

For fuller references see especially Kobert (20 and 28) and Grünfeld (30).

(1) Vauquelin, *Ann. Chim. Phys.*, III, p. 337, 1816. Abstract in *Journ. Roy. Institution*, II, p. 320, 1817.

(2) Pettenkofer, *Repertorium f. Pharm.*, III, p. 48, 1817.

(3) Wiggers, *Prize dissert.* (Latin). Göttingen, 1831. Transl. in *Ann. d. Pharm.*, I, p. 129, 1832.

(4) Bonjean, *Histoire physiologique, chimique, toxicologique et médicale du seigle ergoté.* Paris et Lyon, 1842. (Ref. Kobert).

(5) Wenzell, *Amer. Journ. of Pharmacy*, XXXVI, p. 193, 1864.

(6) Manassewitz, *Mag. Diss.* (Russian); transl. in *Pharm. Zeitschr. f. Russland*, VI, p. 387, 1867.

(7) Herrmann, *Neues Repertorium f. Pharm.*, XX, p. 283, 1869. Abstract in *Chemisches Centralblatt.*

(8) Ganser, *Arch. d. Pharm.*, CXCIII, p. 195, 1870.

(9) Wernich, *Arch. f. path. Anat. u. Phys.*, LVI, p. 505, 1872.

(10) Buchheim, *Arch. f. exp. Path. u. Pharm.*, III, p. 1, 1874.

(11) Zweifel, *Arch. f. exp. Path. u. Pharm.*, IV, p. 387, 1875.

(12) Tanret, *Compt. Rend.*, LXXXI, p. 896, 1875. See also *Ann. Chim. Phys.* [v], XVII, p. 493, 1879.

(13) Yvon, *Bull. gén. de thérap.*, XCIII, p. 79, 1877.

(14) Dragendorff und Podwyssotski, *Arch. f. exp. Path. u. Pharm.*, VI, p 153, 1877.

(15) Blumberg, *Mag. Diss.* Dorpat, 1878. Transl. in *Pharm. Journ.* [iii], IX, pp. 23, 66, 147, 598; 1878.

(16) Gehe and Co., *Handelsbericht*, p. 61, April, 1883.

(17) Podwyssotski, *Pharm. Zeitschr. f. Russland*, XXII, p. 393, 1883.

(18) Chahbazian, *Bull. gén. de thérap.*, Jan. 15, 1884.

(19) Denzel, *Arch. d. Pharm.*, CCXXII, p. 49, 1884.

(20) Kobert, *Arch. f. exp. Path. u. Pharm.*, XVIII, p. 316, 1884.

(21) Tanret, *Journ. Pharm. Chim.* [v], XI, p. 309, 1885.

(22) Erhard, *Centralbl. f. Gynäk*, X, p. 309, 1886.

(23) Graefe, *Centralbl. f. Gynäk*, X, p. 529, 1886.

(24) Lewitsky, *Inaug. Diss.* (Russian). St. Petersburg, 1887. Abstract in *Hist. Stud. a. d. pharm. Instit. z. Dorpat*, 1889. (Ref. Kobert).

(25) Kobert, *Arch. f. exp. Path. u. Pharm.*, XXII, pp. 90 and 101, 1887.

(26) Bombelon, *Pharm. Zeitung*, XXXIII, p. 109, 1888. (Ref. Kobert).

(27) Thomson, *Centralbl. f. Gynäk*, XIII, p. 172, 1889.

(28) Kobert, *Real-Encyklopädie der gesammten Pharmacie*, 1889. Article, 'Mutterkorn.'
(29) Voswinkel, *Pharm. Central-Halle*, XII, p. 531, 1891. (Ref. Grünfeld).
(30) Grünfeld, *Arbeiten d. pharm. Instit. z. Dorpat*, VIII, p. 109, 1892.
(31) Kruskal, *Arbeiten d. pharm. Instit. z. Dorpat*, VIII, p. 170, 1892.
(32) Keller, *Schweiz. Wochenschr f. Chem. u. Pharm.*, XXXII, p. 121, 1894.
(33) Tanret, *Journ. Pharm. Chim.* [v], XXX, p. 229, 1894.
(34) Ehlers, *L'ergotisme.* Masson et Cie, Paris, no date (after 1894).
(35) Keller, *Schweiz. Wochenschr. f. Chem. u. Pharm.*, XXXIV, p. 65, 1896.
(36) Zutz, *Arch. f. exp. Path. u. Pharm.*, XXXVIII, p. 414, 1897.
(37) Jacobj, *Arch. f. exp. Path. u. Pharm.*, XXXIX, p. 104, 1897.
(38) Meulenhoff, *Prize dissert.* (Dutch) *Ber. van de Ned. Maatsch. ter bev. d. Pharm. Achtste Volgreeks*, No. 1, 1899.
(39) Santesson, *Skand. Arch. f. Physiol.*, XIII, p. 107, 1902.
(40) Plumier, *Journ. de phys. et pathol. gén*, VII, p. 13, 1905.
(41) Sollmann and Brown, *Journ. Amer. Med. Assoc.*, July 22, 1905.
(42) Dixon, *Lancet*, I, p. 827, 1906.
(43) Dale, *Journ. of Physiol.*, XXXIV, p. 163, 1906.
(44) Vahlen, *Arch. exp. Path. u. Pharm.*, LV, p. 131, 1906.
(45) Barger and Carr, *Chem. News*, XCIV, p. 89, 1906.
(46) Kraft, *Arch. d. Pharm.*, CCXLIV, p. 336, 1896.
(47) Barger and Dale, *Arch d. Pharm.*, CCXLIV, p. 550, 1896.
(48) Meltzer and Auer, *Amer. Journ. of Physiol.*, XVII, p. 143, 1906.
(49) Tanret, *Journ. Pharm. Chim.* [vi], XXIV, p. 397, 1906.
(50) Cushny, *Journ. of Physiol.*, XXXV, p. 1, 1906.
(51) Barger and Carr, *Journ. Chem. Soc.*, XCI, p. 337, 1907.

Note added May 8th, 1907.—We have recently been in private communication with Prof. Vahlen, who is kind enough to inform us that he has been continuing his chemical examination of Clavin, and agrees that one of the constituents may be an amino-caproic acid. With regard to the other constituent, yielding the more soluble copper salt, he has departed from his original view that it was also a nitrogenous acid, and now holds that it is not an acid, and that it is the active substance.