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ERGOT AND ITS ACTIVE PRINCIPLES.

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Although ergot has long been established as one of the most valuable drugs at the disposal of the physician, its pharmacology remained, until the last few years, in a state of uncertainty and confusion.

Several causes contributed to this unsatisfactory result. In the first place the systematic position of ergot, as a fungus, rendered the chemical isolation of its active constituents a matter of peculiar difficulty. The search was further complicated by the fact that the most diverse opinions prevailed as to the type of physiological action which should be regarded as characteristic of a principle to which the therapeutic effect of ergot could be attributed. To some extent this difficulty still prevails. Not a little of the confusion, again, was due to the almost reckless manner in which successive observers bestowed names suggestive of chemical individuals on the crudest of extracts, or renamed substances isolated by their predecessors, through failure to compare their own results with those already published. As instances of these tendencies may be cited, on the one hand, the name "Ergotin" still used especially in German literature, which was assigned in turn by Wiggers, Bonjean, Wenzell, Wernich, Yvon and others, in each case to a quite different kind of crude extract; and, on the other hand, the names picrosclerotine, and secalin, given by different observers to the one ergot alkaloid which, at that time, had been obtained in pure condition, and which Tanret, who first isolated it, had named "ergotinine."

This isolation of ergotinine by Tanret, in 1875, may be regarded as the first step of real importance towards the recognition of the specific active principle of the drug. It is remarkable, indeed, how nearly the problem was solved at this comparatively early date in the history of its investigation. Tanret's ergotinine has been found, as already mentioned, by several subsequent observers, and its formula has now been definitely settled by the analyses made by Barger and Carr, whose correction of that originally given has been confirmed by Tanret himself. In one respect Tanret's results have not been substantiated by recent work. Finding that ergot yielded, in addition to the easily crystallizing alkaloid, a further quantity of alkaloid giving practically identical chemical reactions, but refractory to crystallization, Tanret regarded this latter as an amorphous form of the same alkaloid.

In this assumption he was undoubtedly in error, the amorphous alkaloid being, indeed, closely related to, and easily formed from the crystalline, but not chem-

ically identical with it. The failure to recognize this difference, though a small point in itself, had a far-reaching effect on the pharmacological history of the drug. On the basis of clinical results, obtained either with the amorphous alkaloid, or with acid solutions of the crystalline alkaloid, in which the amorphous is rapidly formed, Tanret concluded that he had isolated the active alkaloid of ergot. When, however, his alkaloid was subjected to pharmacological experiment by Kobert, the crystalline ergotinine as being that of which the purity could be guaranteed, was naturally taken, and injected in fresh solution. Kobert rightly concluded that it has practically no activity; and since, according to Tanret, the amorphous alkaloid was chemically identical with it, ergotinine was dismissed as of no pharmacological interest, though it still retained some vogue in practical therapeutics. As a result the chemical investigation of the drug was again given over to the preparation and testing of crude resinous products, though the work of Kobert in particular did something towards determining the manner which the specific toxic effects could be recognized. Kobert claimed to have separated from ergot three principles, which, though not chemically pure, had each a separate and distinct physiological action. One of these, "ergotinic acid," was admittedly of no therapeutic interest, having an action which Kobert regarded as related to that of saponins, and need not be further considered. A second, "cornutin," a resinous alkaloid preparation, was found to produce convulsions in frogs and mammals, and was regarded by Kobert as responsible for the convulsant type of ergotism, prevalent in most of the epidemics of ergot-poisoning in northern and eastern Europe. The nature of the substance producing this convulsant action, in Kobert's experiments, is one of the points in the pharmacology of ergot which still remains obscure. According to the method of preparation, "cornutin" must have contained the alkaloid now known as "ergotoxine;" but this latter does not possess the peculiar action in question. Subsequent observers have failed to obtain from ergot an alkaloid possessing this action, and it is admitted by Kobert and his pupils that their own recent attempts to obtain it have not succeeded. The preparations commercially obtainable under the name "cornutin" consist of more or less impure and resinfied mixtures of the known ergot alkaloids, and have not the marked convulsant action. Whether Kobert was dealing with a peculiar decomposition product or with an alkaloid occurring exceptionally in the batch of ergot with which he worked will probably never be settled; in any case "cornutin" cannot be regarded as a chemical entity or a normal ergot constituent. Kobert's third active principle was an acidic resin, named "sphacelinic acid." This was found to produce the gangrene which formed the predominant feature in the epidemics of ergotism in France, as well as a well-marked gastro-intestinal inflammation. Experimentally the symptoms were seen most typically in fowls. Jacobj's experiments were directed to a closer chemical characterization of the active principle of Kobert's sphacelinic acid. By ethereal extraction he obtained from ergot a yellow substance, producing gangrene in fowls, to which he gave the name "chrysotoxin." By extraction with organic acids he separated from this an alkaloidal fraction consisting of a crystalline inert alkaloid, undoubtedly identical with Tanret's "ergotinine," but called "secalin" by Jacobj, and an amorphous substance, of high physiological activity,

which he called "sphacelotoxin." This latter, for reasons which now appear to be inadequate, Jacobj described as a non-nitrogenous resin, in spite of the fact that his analyses of "secalintoxin" (i. e. "secalin"+"sphacelotoxin") and of secalin (=ergotinine) show identical percentages of nitrogen.

With regard to the therapeutic bearing of these investigations, Jacobj regarded sphacelotoxin as the bearer of the therapeutic as well as the toxic properties of ergot. Kobert, at one stage of his investigation, attributed the therapeutic effect on uterine activity to "sphacelinic acid," at another to "cornutin." Probably the main effect of these researches on the course of ergot investigations was the establishment of the cock's-comb test as an empirical measure of the activity of the drug. From the chemical point of view the subject was left, in 1897, in a far worse position than that to which Tanret had brought it in 1875, and the official pharmacological teaching concerning ergot became once more a matter of complicated terminology for ill-defined substances.

This was the state of affairs when, in 1904, an investigation was begun at the Wellcome Physiological Research Laboratories. Those responsible for this soon came to the conclusion that it would be fruitless, in the first instance, to search for a principle endowed with all the physiological actions which, at one time or another, had been attributed to ergot, and associated, on inadequate evidence, with its therapeutic value. The first step, rather, must be to take some characteristic action, which could be regarded as probably the effect of one active constituent, and endeavor to ascertain the nature of that constituent. It would then be possible, if a chemically pure principle were obtainable, to investigate its relation to other types of action, and to search further for other principles, if it became clear that more than one was involved. It seemed natural, at that stage in the history of the drug, to start by examining and further analyzing the action of preparations made according to the methods of Kobert and Jacobj. The results of this preliminary investigation are embodied in the first paper by H. H. Dale, in which a highly characteristic effect on the function of the true sympathetic system is described. All the preparations tested had a potent stimulating action on plain muscle, succeeded by a paralysis of motor sympathetic effects, while the inhibitor actions of the same system were left unaffected. The most readily observed instance of this action was the fall of blood-pressure resulting from injection of the suprarenal active principle, in place of its normal, typical, pressor action. It may be noted, in passing, that this so-called "vasomotor reversal" test has been the subject of some criticism by those who have attempted to use it as an indication of ergot-activity in general. It is desirable to make it clear that its originators never claimed for it any value except as a test for a particular active principle, and that it is not surprising that others have failed to find it applicable to extracts owing their activity chiefly to other substances. The search for the substance producing this action was conducted by G. Barger of the Wellcome Physiological Research Laboratories, working in conjunction with F. H. Carr of the Wellcome Chemical Works, Dartford, their results being constantly controlled by Dale's physiological experiments. They were soon able to identify the active substance as an alkaloid, closely resembling Tanret's ergotinine in many of its chemical properties, but differing from it in solubility and in the

fact that it could not be crystallized as a free base. They were able to obtain it pure however, in the form of its salts, many of which crystallize readily. In this respect, again, it differs from ergotinine, the salts of which have resisted all attempts to crystallize them, though the base itself crystallizes well. A physiological examination of the pure salts of the new alkaloid showed that it not only possessed the characteristic type of action which afforded the clue to its isolation, but produced typical gangrene of the cock's-comb and the other toxic actions ascribed by Kobert to "sphacelinic acid" and by Jacobj to "sphacelotoxin." To this active amorphous alkaloid, with crystalline salts, the name "ergotoxine" was given by Barger and Carr.

By one of the not infrequent coincidences of scientific work F. Kraft, who had also for some years been working at the chemistry of ergot, arrived almost simultaneously at the conclusion that ergot, in addition to Tanret's crystalline ergotinine, contained a second, amorphous alkaloid. The results of his investigation, which also threw much light on the chemistry of some of the inactive constituents of the drug, were published only one month after Barger and Carr's preliminary note, which had escaped his notice. His separation of the two alkaloids was based on the different solubilities of their sulphates. Since he obtained these only in an amorphous form, there was no guarantee that the separation was complete, and he made no analyses. At the same time, on the basis of observations as to the methods by which each alkaloid could be converted into the other, he put forward the suggestion that the amorphous alkaloid was a hydrate of the crystalline ergotinine, and, being unaware that it had been named just previously by Barger and Carr, who provisionally named it "hydro-ergotinine." Having crystalline salts of their alkaloid fit for analysis, Barger and Carr were then able to confirm Kraft's suggestion as to the relation between the alkaloids, and Kraft himself subsequently obtained crystalline salts by their method, and further confirmed the identity of his alkaloid with theirs and its relation to ergotinine. The chemistry of the ergot alkaloids being thus placed on a satisfactory footing by the concurrent though completely independent work of two laboratories, it became of importance to examine the relation of these alkaloids to previously described "active principles." As the result of a lengthy investigation, Barger and Dale came to the conclusion that the "amorphous ergotinine" of Tanret consisted largely of the alkaloid now known as "ergotoxine;" that the crystalline ergotinine was, in reality, inert, and only appeared to possess activity on account of the readiness with which, in watery acid solution, it became converted into its intensely active hydrate "ergotoxine;" that preparations such as "sphacelinic acid," "chrysotoxin," "sphacelotoxin," owed all their activity to the presence of ergotoxine in greater or less proportions. It should be noted that, since ergotoxine has weak acid as well as weak basic properties, and since its salts, moreover, form colloidal solutions in water, its presence as an activity-conferring constituent in acidic resins is easily explained. Barger and Dale published a table of synonyms, indicating the importance of ergotoxine as the active constituent of the various principles described up to that date. At the same time they recognized and, indeed, explicitly stated, that certain features of the action of some of the most widely used extracts of ergot could not be accounted for by the presence

of ergotoxine. The fluid extract of the U. S. P., being an acid alcoholic extract, contains, indeed, a large proportion of the ergotoxine of the ergot from which it is made, and doubtless owes to this a great part of its therapeutic value. Edmunds and Hale recently arrived at the conclusion that the effect of this extract on the uterus runs parallel to its activity as determined by the cock's-comb test, which they rightly regard as a measure of its ergotoxine value. On the other hand, such preparations as the "Extractum Ergotae Liquidum" of the British Pharmacopoeia, which has a great vogue among practitioners in Great Britain, usually contains mere traces of ergotoxine. Yet this extract exhibits two definite types of physiological action, which have been recommended by different authorities as measures of its therapeutic value—it has a pressor action, of the adrenine type, and it causes pronounced contraction of the isolated uterine muscle. Barger and Dale proceeded to investigate the nature of the substances responsible for these types of activity. Shortly before this, Vahlen had announced the discovery in ergot of an active principle with no toxic properties, but possessing a specific stimulating action on the normal, coordinated contractions of the pregnant uterus. To this principle he gave the name "clavin," and was soon able to cite clinical evidence in favor of its activity. Barger and Dale examined this preparation and found it to be a mixture of aminoacids and quite devoid of activity. Their statement as to its chemical nature was confirmed by Van Slyke, who separated it into leucin, isoleucin, and valin, and determined the proportion of each which was present. Several other observers (Cushny, Kehrer, Cronyn and Henderson) had also found it inactive. Vahlen's results are, therefore, of interest only as evidence of the great difficulty in obtaining and interpreting clinical evidence as to the effect of drugs on uterine activity. It became necessary to look elsewhere for the active constituents, other than ergotoxin, of which the existence was evident. For a long time no success was obtained, for the principles in question could not be removed from ergot extracts by any of the methods ordinarily employed for the isolation of alkaloids. It seemed possible, however, that ergot, being a fungus, might resemble the bacteria rather than the higher plants in its metabolic processes, and that an investigation of physiologically active substances produced by the putrefaction of proteins might furnish a clue to the nature of the other active ergot constituents, and suggest methods for their isolation. A resemblance between ergot constituents and the products of putrefaction was, indeed, suggested by Buchheim as long ago as 1874. But the significance of this suggestion, as of Tanret's work on the alkaloids, which followed a year later, had been obscured by the investigations of the intermediate period. Barger and Walpole accordingly studied the pressor constituents which occur in putrid muscle extracts, as Abelous and his pupils had previously shown. They found them to belong to the series of amines, formed from amino-acids by splitting of carbon dioxide, the most abundant being isoamylamine (from leucine), the most active p. hydroxyphenylethylamine (from tyrosine). Dale and Dixon showed that the action of these bases is of the same general type as that of the supra-renal active principle. Meanwhile, the probability that the other active constituents of ergot were to be sought in this direction was increased by the work of Rielander who extracted from ergot the well-known but almost inactive

bases putrescine and cadaverine. Applying the experience gained with putrid meat, Barger was able to prove the presence of the pressor amines in the ordinary liquid extract of ergot, and to show, in conjunction with Dale, that practically the whole of the adrenine-like pressor action possessed by such extracts, and widely used in England at the time as a basis for their physiological standardization, was due to the presence of p. hydroxyphenylethylamine. This substance has been produced artifically by a number of synthetic methods and is now obtainable commercially under the name "Tyramine."

The investigation was not yet complete, for "Tyramine" was found to resemble adrenine not merely in its pressor action, but in practically all its effects, reproducing very closely the effects of stimulating nerves of the true sympathetic system. Among such, a highly characteristic action is the inhibition of the tone and rhythm of the uterus of the virgin cat, and this is typically reproduced by "Tyramine." On the other hand, it has been shown by Kehrer that isolated uterine muscle from any animal responds by tonic contraction to small doses of ergot extracts, and that the uterus of the non-pregnant cat exhibits this effect particularly well. Though ergotoxine has a powerful tonic effect on the cat's uterus *in situ,* it has a comparatively weak action on the isolated organ; it was clear, therefore, that some other principle must be present of sufficient power in this direction to overcome the inhibitor effect of tyramine. In searching for this, Berger and Dale made use of the methods elaborated by Kutscher and his school for the separation of bases from extracts of meat or putrefation mixtures.

They chose for the investigation the ergot extract which produced Kehrer's effect most intensely, viz. the "Ergotinum Dialysatum" of Wernich. In the end they succeeded in isolating a small quantity of the crystalline picrate of a base which, while it resembled histidine in its properties of solubility and precipitation by reagents, and gave the diazo reaction of Pauly with great intensity, differed from histidine in producing Kehrer's effect in extreme dilutions, whereas histidine is quite inert in this direction. It was a natural supposition that the base might bear the same relation to histidine as "tyramine" to "tyrosine." By another of the curious coincidencies which have occurred in the course of this investigation, the same base had been obtained from ergot by Kutscher simultaneously and independently. At the same time Ackermann, by putrefaction of a broth containing histidine, had obtained a supply of the base which results when histidine loses CO_2. Barger and Dale found the histidine derivative identical with their ergot base, while Ackermann and Kutscher concluded that the two were similar but not identical. It has since been shown that the apparent difference in physiological action, on which Ackermann and Kutscher based their conclusion, was due to an unsuspected variation of the conditions of experiment, and it may be regarded as established that the principle in ergot mainy responsible for its intense tonic action on isolated uterine muscle is b. Iminazolylethylamine (i. e. histidine minus CO_2). This is now prepared synthetically, and obtainable in commerce as "Ergamine." Its action has been investigated and described by Dale and Laidlaw, who have drawn attention to the interesting similarity between the effects which it produces on intravenous injection, and those which follow the

injection of various tissue extracts and form the main feature in the clinical picture of the "anaphylactic shock." At the same time it has been demonstrated that the base can be given hypodermically in small doses without producing bad symptoms, but with marked effect on the uterus.

Engeland and Kutscher subsequently isolated from ergot agmatine, the analogous amine from arginine, and attributed to it a similar action. According to Dale and Laidlaw the effect of agmatine is very weak, and it cannot contribute in any significant degree to the action of ergot.

It is not suggested, of course, that all the constituents possessing a physiological action of any kind, which occur in any sample of ergot or its extracts, have been isolated and identified. On the contrary, it has been obvious, from the latter portion especially of Barger and Dale's work on this subject, that the casual occurrence of unusual bases is only to be expected in extracts of a fungus which shows many similarities in its action to certain putrefactive bacteria. It is even uncertain, in any particular case, how much of the active amine constituent must be attributed to the metabolism of the ergot itself, how much to superadded putrefactive changes occurring either before or during extraction. What is quite certain is that good, dry ergot contains not merely ergotoxine, but also the bases in question, though the proportion of these latter may well be increased in the preparation of such a product as the Liquid Extract of the B. P., and still more so in the dialysed preparations. It may further be claimed that the substance responsible for each of the various physiological actions, hitherto suggested as a basis of standardization, has been isolated and identified. The effect on the cock's-comb was given practical application for standardization by Houghton, who gave details by observance of which a quantitative indication was obtainable. This has been recently verified by Edmunds and Hale. This action, like the "vasomotor reversal" described by Dale, is due to ergotoxine, and while the practice of different observers may lead them to prefer one or another of these methods, they are testing, in either case, for ergotoxine only. When the measurement of pressor effect, in dog or cat, as recommended by Wood and by Cronyn and Henderson in America, and by Dixon, Goodall and others in England, is adopted as an index of activity, the measurement appears to be one of ergotoxine +"tyramine" in the case of the Liquid Extract of the British Pharmacopoeia, Kehrer's method, again, if a cat's uterus be used, is apparently a measurement of "ergamine" content only; on the other hand, the isolated guinea-pig's uterus is exquisitely sensitive to ergotoxine as well as to ergamine." It will doubtless be possible by a combination of methods to work out a rational system of ergot standardization when it is once decided which of the principles are therapeutically desirable and in what relative proportions they should occur. What is needed above all for the settlement of this aspect of the ergot-problem is an accumulation of accurate clinical observation with the pure active principles. Meanwhile it may be said that results hitherto available appear to point to the superiority of a preparation containing the three chief active principles in appropriate proportions, as compared with any one of them separately. It should be further remembered that ergotoxine is the only one of them for which ergot is needed. The others are much more easily obtained by synthesis in the laboratory, and it may be as-

sumed that the ideal preparation is one containing a definite quantity of pure ergotoxine from ergot, with the amines added in due amount. A preparation containing 1 mgm. of ergotoxine to 5 mgms. of "Tyramine" and 0.05 mgms. of "Ergamine" has given highly satisfactory results in practice. But pending more decisive clinical information it may be suggested that no one method of standardization can yet claim exclusive value or unquestioned superiority, whatever be its accuracy in determining the proportion of one or more of the active principles.