REVIEW ARTICLE

THE INFLUENCE OF THE SOIL ON THE CONTENT OF ACTIVE PRINCIPLES IN MEDICINAL PLANTS

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Introduction

The active principles of vegetable drugs are products of the metabolism of the producing plants. It may therefore be convenient to begin this review with a very brief exposition of the role of the main groups of active principles in plant metabolism. Unfortunately it must be said that our knowledge on these problems is still rather poor. This becomes evident on reading the excellent book recently published by Paech[1]. In spite of a rather abundant number of individual investigations only a few conclusions of a more general character have been made about the physiological role of the active principles. These conclusions may be summarised as follows.

The *alkaloids* are products of nitrogen metabolism. As James[2,3] has recently shown in an excellent manner, no satisfying explanation of their significance for the plant metabolism exists. They may be produced either in the anabolic or katabolic processes[4].

The *glycosides* are connected with carbohydrate metabolism by their sugar components. On the other hand, the aglycones which belong to different classes of chemical substances, must originate from different metabolic processes, partly even from the nitrogen metabolism (mustard oils, hydrocyanic acid). Others, like steroid aglycones, may originate from processes similar to that now to be discussed for essential oils.

Resins and Essential Oils. The question whether they are produced from side reactions of carbohydrate metabolism or from nitrogen metabolism is still not quite settled, although Paech[1] is strictly against any connection with the nitrogen metabolism. He believes, that these substances, which may for their major part be reduced to open or cyclic terpenes, are built up from comparatively uncomplicated substances like acetone, acetaldehyde and methylcrotonaldehyde. Paech designates the alkaloids, glycosides and essential oils (including resins) as products of side reactions of plant metabolism and James also considers them, not as waste products, but as products of side reactions, specific for a few species of the plant kingdom.

Mucilages and Gums. These originate from carbohydrate metabolism. Jaretzky[5] considers them to be reserve material, which can be easily mobilised. On the other hand, due to their retaining capacity for water, they may have some significance as accumulators of water. The gums, which are predominantly formed after injury or microbial damage of the tissues, could also be considered as a kind of protective product.

ERRONEOUS FINDINGS

On looking through the great number of publications dealing with our subject, one is astonished that for many problems contradictory results have been obtained or at least that the results have been interpreted with contradictory meanings. Recently, Hegnauer[6] has drawn attention to this fact. Such contradictory results may occur for different reasons and it seems necessary to summarise the most important of these reasons before beginning the exposition of the subject itself.

(1) A first source of erroneous findings arises from the fact that the active principles are products of metabolism and therefore the results of very complicated processes. These processes are influenced by internal factors which may vary in every strain of a species (genotypical factors) and by external factors (such as soil, climate, neighbouring plants, etc., generally called phenotypical factors). It is a well-known fact that in a certain measure an external factor may be replaced partly by another one (e.g., light by temperature). From this possibility of replacement and interaction of external factors it follows, that it must be very difficult, or even impossible, to demonstrate with full certainty that a measured effect has been provoked by a single definite factor, especially in field trials.

(2) A further source of errors is caused by the fact that plants, even of the same genetical strain, will react differently according to their state of nutrition and to former influences. As an example, Cromwell[7] demonstrated that potassium nitrate produced an increase of the alkaloidal content of belladonna only if the plant had a sufficient supply of carbohydrate, and that a lack of such provision produced a decrease with the same nutrient. Again, with field trials, it is impossible to control sufficiently such a supply and errors may be produced by unequal supply.

(3) Another source of errors lies in the seasonal, diurnal and ontogenetic variation of plant metabolism and especially in the variation of the content of active principles. Care should therefore be taken to collect the plants at the same state of development, at the same or corresponding date and time of day and under the same atmospheric conditions. Such variation may be greater than the variation produced by the factor to be examined. This has not always been considered sufficiently and in many papers no indication concerning this point is made.

(4) The analytical methods used by different investigators often differ widely. Therefore the absolute values of different investigations must be compared critically and it may even be impossible, in some cases, to make any serious comparison.

(5) In many previous papers sufficient consideration has not been given to statistical requirements. Future papers should give sufficient information about such data, e.g., the number of plants and the limits of error of analytical methods.

(6) The reference basis or reference quantity on which the results are calculated is of highest importance in any investigation on the subject discussed here, as has been demonstrated recently by Hegnauer[8].

Commonly, the following reference bases are used: fresh weight, dry weight, crude fibre, calcium content[8] and surface (only for leaves). As during drying at lower temperatures ($<50°$ C.) the respiration of the organ continues and consumes a part of the plasmatic substance, the relation between the active principle determined and the whole plant material is affected. Therefore physiological conclusions should be drawn from such results critically and with the greatest caution. Only the other reference bases mentioned above are suitable for physiological investigations. Nevertheless, in many former publications (including some from our own laboratory) this mistake has been made. On the other hand, it must be considered that many of the investigations have been planned and performed with regard mainly to the practical production of drugs and, for this purpose, dry weight as determined by processes similar to those in practical drug production, is absolutely indicated as a reference basis. In this paper we shall refer, in most cases, only to the content of active principles and not to the biochemical processes occurring during the growth of the drug plants and producing the active principles.

Speaking generally, it would be ideal to investigate only one factor of complexes like soil and climate, all other factors of the complex being maintained constant. Such researches are only feasible in well equipped laboratories. Most previous investigations have not been carried out under such conditions and therefore interaction of the influencing factors has occurred to a greater or lesser extent. Several researches had also the aim to make clear the influence of a well defined complex, e.g., northern or southern or mountain climate, special types of soil, e.g., calcareous or siliceous, light or heavy soil, etc. We shall first give a survey of papers dealing with individual factors and later of those dealing with complexes of factors.

Influence of Individual Soil Factors

Physical Factors. The influence of physical factors has not been widely studied up to the present. The particle size of soils showed some influence on mucilaginous plants. On sandy soils (greater particle size), *Althæa officinalis* yielded roots with higher mucilage content than on clay soils (smaller particle size)[9].

The influence of the moisture of the soil is rather difficult to investigate because the mineral substances essential for growth affect the intake of water. Differences in water content of quartz sand had no significant influence on the percentage of essential oil in dried leaves of peppermint, but caused a slight increase in fresh leaves. On the other hand, the total amount of oil produced per unit of area increased considerably, due to the great increase of the yield of green material[10]. With *Althæa*, Dafert[9] found a decrease of mucilage content concordant with a rise of soil moisture and Rutschkin[11] a decrease in the iodine value of linseed oil concordant with an increase of soil moisture.

Hydrogen ion concentration is, for most of the species, without injurious effect on growth within ranges of 1 to 1·5 *p*H units (*Hyoscyamus niger* 6·4 to 7·9, *Matricaria chamomilla* 7·3 to 8·1, *Chrysanthemum cinerariæfolium*

5·9 to 8·1, *Majorana hortensis* 5·6 to 6·4)[12]. Sandfort[13] mentions a Russian author (Suchurow) who found that *Datura stramonium* grows best at a *p*H of about 7, but that the alkaloidal content is higher in slightly alkaline or acid soils. Again, Birkeli's[10] careful investigations showed that *p*H had a distinct influence on the growth of peppermint on quartz sand between 4·2 and 7·2. No significant influence of *p*H on the essential oil content of peppermint or on the nicotine content of tobacco-leaf could be found[10,14].

Chemical Factors. If investigations on physical factors are still rather scarce, those on chemical factors are, at least for several groups of plants, rather abundant. Most of them have been performed by adding increasing quantities of one nutrient or by excluding it totally, partly on natural soil, partly also on sand or water cultures. It is rather striking, that with sand or water cultures the influence of unilateral additions of nutrients was found rather less effective than on natural soils. An explanation for this finding may be that the plant cannot fully profit from the added nutrient (insufficient intake or assimilation) except in the presence of other, mostly unknown, constituents or physical properties, which are present in natural soil, and help to utilise fully the added nutrients. The greater number of researches deal with the influence of the three main nutrients, nitrogen, phosphorus and potassium, and we shall give the results separately for each element, the plants being arranged according to their active principles. A short general survey of the influence of other elements will be given for all the groups of constituents.

Plants containing Alkaloids.

Nitrogen in the inorganic form or as stable manure produced a significant increase of the alkaloidal content of *Atropa belladonna*[7,15–22], *Hyoscyamus niger*[22,23], *Datura stramonium*[24,25,26], *Datura innoxia*[27,28] and *Nicotiana*[29,30]. Huter[30] demonstrated that in *Nicotiana* ammonia nitrogen produced a greater nicotine content than nitrate nitrogen. In *Datura innoxia* the proportion of scopolamine to hyoscyamine was not affected by fertilisers[28]. Other investigators found no increase, or at least no constant increase, of the alkaloidal content in belladonna[31], stramonium[32] and *Hyoscyamus*[31,32]. With *Lobelia*, in all known investigations, nitrogen considerably lowered the content of total alkaloids[33,34,35,36]. With the opium poppy an increase of opium yield was found by Annet[37], but no increase in the morphine content of the opium[37,38]. In potato tubers, Sabalitschka and Jungermann[39] proved an increase of the glycoalkaloid solanin, whilst Pallmann and Schindler[40] in very careful and statistically calculated trials could find only a very slight increase and that only during the growth period of the tuber.

Phosphorus. The influence of this element on alkaloidal plants has been much less investigated than that of nitrogen. With *Datura*, Maurin[41] found an increase. Gstirner[20] could not detect any alteration of the content of alkaloids for belladonna and other authors[12,42] proved that greater additions of phosphorus lowered the amount of alkaloids in *Hyoscyamus*. *Lobelia* grew better when phosphorus was present in the soil in larger quantities[33,34], but the amount of alkaloids in the dried drugs was lower in such drugs. The bark of *Punica granatum* contained twice as much alkaloids when manured with phosphates as without that nutrient[43], and also in *Chelidonium*, where the alkaloids are part of the latex, their amount was increased by phosphates[44]. The percentage of morphine in opium is not affected by phosphorus[44].

Potassium seems to lower to some extent the alkaloidal content of belladonna[19], stramonium[24], *Datura innoxia*[28], *Nicotiana, Lupinus*[45] and *Punica granatum*[43]. In other investigations higher contents were found in plants manured with potassium for *Hyoscyamus*[23], *Aconitum*[46] and *Solanum tuberosum*[39], and Gstirner[20] found for belladonna no effect with the leaves but an increase of the alkaloidal content of about 30 per cent. for the roots. Bärner[36] and Esdorn[34] detected clear increases, even on sand cultures, for *Lobelia.* The morphine content in opium was not affected by potassium[24].

The *trace-elements* boron, zinc, copper and cobalt had no significant influence on the alkaloidal content of *Datura innoxia*[27], whilst thorium increased considerably the content of alkaloids in the bark of *Punica granatum*[43] and uranium is believed to promote growth, but to lower the amounts of alkaloids, in stramonium[41]. The results with these 5 elements are certainly still insufficient to permit a definite conclusion about their influence on the content of alkaloids to be drawn. Iron gave drugs with higher amounts of alkaloids[41] in *Datura* and in *Lupinus*[45] and was without influence on belladonna.

Plants containing Glycosides.

Digitalis, and, to a somewhat less extent plants containing *saponins* and *mustard oil glycosides*, have been examined.

For *Nitrogen*, Boshart[24] found an increasing effect on the amount of active principles in the seeds of black mustard, whilst Dafert and Thomas[47] could not detect any effect with the same plant. *Digitalis lanata* and *Digitalis purpurea* yield leaves with slightly higher cardiac activities[48,49]. On sand cultures the increase was more pronounced than in the field[49]. An important research by Jaretzky and Seiffarth[50] deals with the influence of different nitrogen compounds on saponin-containing plants. The *hæmolytic activity* of leaves and roots of *Saponaria ocymoides* and of *Lychnis Flos-cuculi*[50] was lowered by 26 to 48 per cent. if the source of nitrogen was ammonium sulphate. With calcium nitrate the activity was increased in the leaves and decreased in the roots. Ammonium chloride gave no alteration of the activity in comparison with the control. Ammonium, sodium and potassium nitrates favoured the growth of both species and potassium nitrate gave drugs with higher activities. This investigation shows in an impressive manner that definite results concerning the influence of nutrients on the activity of vegetable drugs can only be established if different compounds of the nutrient have been studied. With *Saponaria officinalis* nitrogen increased the hæmolytic activity only in seedlings, whilst with scions and older plants no effect was obtained[51]. This finding is in concordance with the general point of view, that most of the active principles are predominantly produced in growing, young tissues.

Phosphorus caused with *Digitalis lanata* in sand cultures, a significant increase of the cardiac activity[49]; in normal soil the increase caused by phosphorus was rather small or negligible, or even a small decrease resulted[48,49] The saponin content of *Saponaria officinalis* was not affected by phosphorus fertilisers.

Potassium caused a lower content of sinigrin in the seeds of *Brassica nigra*, whilst in the roots of *Raphanus* a higher percentage of this glycoside was found[38]. *Digitalis lanata* was found to contain more glycosides if manured with potassium on sand cultures, while this value decreased if the potassium treatment was given to plants in natural soil[49]. In opposition to this result, Boshart[38] found higher activity with a potassium treatment in natural soil.

Trace-Elements. Manganese was considered to be important for *Digitalis purpurea*, due to the fact that the leaves contain rather considerable amounts of this element. Recently, Duquénois and Schaerrer[52] have shown that there is no proportional relation between the manganese content of the leaves and that of the soil on which the plant had grown. In open land, *Digitalis purpurea* treated with manganese showed in one research[38] a slight increase and in another[53] no definite influence on the cardiac activity of digitalis leaf. Finally, iron and copper have been found to be without action on the pharmacological potency, when given individually or in combination and even in toxic concentrations[54]. This last result is a good confirmation of the lack of influence of iron and copper. The trace elements boron and manganese[50] stimulate the growth of *Lychnis Flos-cuculi* and *Saponaria ocymoides*; but they do not affect significantly the hæmolytic activity. If any inference can be derived from the results it would be that these elements have a tendency to lower the saponin content.

Plants containing Essential Oils.

The most cultivated species is peppermint and it is with this species that the greater number of investigations deal.

Nitrogen. For the influence of nitrogen, again the results of two careful researches on sand cultures come to somewhat different conclusions; Birkeli[10] found that nitrogen produces a healthy and vigorous growth but does not affect the percentage of volatile oil, and Schratz and Wiemann[55] who tried 5 different doses of nitrogen each in 3 parallel series for 2 years had with the optimal dose a maximal increase of 80 per cent. in the oil content. 3 further investigations[56,57,58] on normal soil gave similar results with regard to nitrogen; Schlemmer and Springer[59] again found the highest content without nitrogen (only phosphorus and potassium). This demonstrates the influence and the importance of the character of the soil on which such investigations are performed. Nevertheless, for most of the soils a reasonable dose of nitrogen may be expected to raise the oil content in peppermint. The composition of peppermint oil seems to be less affected by varying the proportions of the main nutrients[59]. *Thymus*, *Basilicum* and *Satureia hortensis* amongst the Labiatæ showed no significant variations of the oil content if nitrogen was under- or overdosed[60]. *Majorana* produced oil contents proportional to the doses of nitrogen[60]. *Coriandrum* and *Pimpinella anisum*[61] responded to high doses of nitrogen with higher oil content in the fruits, and *Carum carvi*[62] and *Valeriana officinalis*[20] did not react to nitrogen fertilisers with regard to the oil content. *Matricaria chamomilla* was found also to give an increased[61] or a decreased[63] oil content, and in the latter case simultaneously the azulene percentage was lowered.

Phosphorus produced increases of the oil content of *Mentha piperita*[55,56,58], while Birkeli[10] found it to be without significant influence. *Valeriana*[20,38,64], *Coriandrum*[61] and *P. anisum*[60] responded with greater or smaller rises in their oil content.

Potassium produced no significant influence with *Mentha piperita*[10,55], but a significant increase with *Valeriana*[20,38] and *Matricaria chamomilla*[65] and a slight decrease with *P. anisum* and *Coriandrum*[61].

The *trace-elements* boron, cobalt, aluminium, tin and copper were found to be absolutely necessary for the growth of *Mentha piperita*[66] because the absence of any of them provoked serious disturbances in the regular growth of the plant.

Effect of Combinations of Nutrients

In many of the researches already discussed here the nutrients have also been given in certain combinations. This is especially necessary for cultures on artificial media (sand or water cultures). It is to be expected that the effect of such combinations on the content of active principles is not only additive, but that some synergistic effects occur. As far as we know, no research has, until now, dealt in detail with the special question of the interaction of the nutrients in relation to the quantitative proportions of such mixtures. We believe that before approaching that question, the influence of individual nutrients should be studied first. Good examples of the complicated conditions arising from such combinations are the papers of Birkeli[10] and Schratz and Wiemann[55].

As a general result most of the plants require a complete and equilibrated mixture of the main nutrients. Only in such media do the plants grow vigorously, and as a matter of fact it is only in fully-developed plants that the content of active principles is satisfactory.

Influence of Natural Soils

If the results of researches with individual soil factors are important both for theoretical knowledge and for practical drug cultivation, experiments with natural soils will also have their value with regard to theoretical knowledge, but they may also be at least as important for practical drug cultivation and especially for the collecting of drugs from wild plants in natural sites. It seemed, therefore, to be of great interest to examine how drug plants behave on such natural soils with regard to their content of active principles.

Only a few results concerning this problem are scattered throughout the literature. The objection which must be made to nearly all these is, that they refer to different soils, situated in different climates, and that therefore the climatic factors may have influenced the plants at least as much as the edaphic ones. Some years ago a research field of the Federal Station for Forestry Research was placed at our disposal. On this field, within a maximal distance of about 100 m., 9 different natural soils from various regions of Switzerland had been established with areas of about 30 to 60 sq. m. and with a depth of about 80 cm. to 1 m. The plants from these plots are therefore grown under absolutely the same climate. The soils show a wide variation with regard to physical and chemical properties. The provision of nitrogen was moderate (4 soils) to very rich (1 soil), of phosphorus, moderate (2 soils) to normal (7 soils), of potassium, poor (1 soil) to moderate (6 soils) and of calcium, poor (3 soils) or rich (6 soils). The *p*H varied from 6·6 to 7·4. The percentage of particles smaller than 2 mm. varied from 38·6 to 95·6 per cent. The average temperature of the soils, determined by the elegant method of Pallmann *et al.*[67] was for a period from July to October at 5 cm. depth from 17·3° to 20·8° C. and at 25 cm. depth from 16·1° to 18·4° C. for the different soils. On these soils we grew during several years medicinal plants, with the collaboration of Wüst[68] and Hoffmann[69]; each species of plant was under test for 2 to 4 years; individual plants were analysed for their content of active principles. As far as we know these are the only comparative investigations on different natural soils in the same climate and we shall briefly report the main results. It seems desirable that the following results of our own researches should be considered separately from other investigations in which both soils and climates were the variables. It may be noted also that Hoffmann[69] has collected from natural sites several of the species cultivated by ourselves.

Plants containing Alkaloids.

For belladonna[69] the soils rich in nitrogen produced the drugs with the highest contents of alkaloids. *Lobelia*[67] behaved in the same way. Phosphorus had for both species, belladonna and *Lobelia*, a favourable effect and the soils rich in phosphorus produced also the highest alkaloidal contents. One soil which was poor in phosphorus produced, nevertheless, a high alkaloidal content in both species. Potassium had rather the opposite effect on the content of alkaloids in both species, a finding which is contradictory with the results of *Lobelia* on manured soil[34,36]. Soils with a high content of soluble potassium produced the drugs with the lowest alkaloidal content, especially with belladonna. Ferric oxide and aluminium trioxide (so-called sesquioxides) had a similar, but less marked, effect to potassium.

Plants containing Essential Oils.

Mentha piperita produced the highest oil contents on soil rich in nitrogen. To a certain extent *Valeriana*[68] showed the same behaviour, whilst in *Pimpinella magna* roots no proportionality between nitrogen in the soil and oil content could be detected. *Artemisia laxa*[69] showed almost no concordance between nitrogen in the soil and oil content; nevertheless, a slight tendency towards higher oil content on soil rich in nitrogen might be noted. *Peucedanum ostruthium* rhizomes[69] were not significantly influenced by nitrogen in the soil. With phosphorus and potassium no clear and significant concordance was found. For *Peucedanum* the drug from the soils with high calcium content had the highest oil content. For peppermint also the isolated oils were analysed. Between the soil factors and the proportion of free and esterified menthol no concordance could be detected. The range of variation of these components may be of some interest and we give one example: the amount of free menthol varied for 1 crop on the 9 soils from 45·8 to 58·3 per cent. On the examination of the volatility on a filter-paper the odour of several oils became unpleasant after about 24 hours and for others it remained pleasant for 96 hours. From one soil (humus), which produced the highest percentage of oil, the odour was strong but not pleasant, and the taste reminded one of wet moss. This demonstrates that the soil yielding the greatest crop and producing the drug with the highest percentage of oil is not always the most appropriate for practical drug cultivation.

Plants containing Carbohydrates.

In *Althæa officinalis* the roots only produced significant differences in the viscosity of the aqueous extract (as a measure of the mucilage content), varying from 1·5 to 4·0 centipoises. The mucilage content of the leaves was nearly unaffected. No definite concordance with nitrogen, phosphorus and potassium was detectable, although the greater part of the more viscous drugs originated from soils rich in phosphorus and nitrogen. On the other hand, physical factors seem to be important for the production of drugs rich in mucilage.

Plants containing Tannins.

In *Bergenia delawayi* the tannin content varied in the wide range from 3·5 to 10·0 (this being the widest variation caused by natural soil in one climate). No concordance with the soil factors under test was evident. As the plants had given similar results during 2 to 4 years, it can be concluded that the variation of the content of active principles from soil to soil remained fairly constant each year. For the evaluation and standardisation of vegetable drugs the range of variation of the content of active principles, due to the soil, must be of great interest. Taking into consideration only drugs from plants, which had not suffered from toxic or starvation effects, the range of variation of the content of active principles is mostly much lower than 100 per cent. and in most of the cases lower than 40 per cent., calculated with reference to the lowest figure. For the tannin content in *Bergenia delawayi*, variations up to 180 per cent., due exclusively to the soil, have been stated[69]. Salgues[46] has also cultivated several plants on natural soils of different types (especially of the calcareous and the siliceous types) and has published a large number of analyses. Unfortunately the cultures on the different soils had been performed in different years and no detailed chemical analyses of the soils are given; for this reason a discussion and evaluation of these results would be very difficult.

CONCLUSIONS

It would have been attractive to give a statistical analysis of several groups of the numerous results set out in the previous pages. For reasons which we have expressed in the introductory notes, we believe that to be impossible, mainly because each investigation had a different experimental basis. From all the results only a few striking findings and general statements will be summarised.

1. Differences in the results of investigations on the same problem and with similar methods (e.g., on sand cultures with mineral nutrients) may be caused by the use of different compounds of the element under test[55], by differences of climate, or by differences in the manner of application of the nutrient (in one or in several doses)[55].

2. Experiments on natural soils with addition of fertilisers may easily give discordant results, due to the variation of the properties of the natural soils (e.g., absorbent capacity, *p*H, buffering capacity, etc.).

3. Favourable effects on the percentage of active principles follow the rule of the optimum; i.e., the increase comes to a maximum (optimum dose) and is then changed into a decrease. This is the point at which the compound becomes toxic[25,55].

4. Of the nutrients nitrogen, phosphorus and potassium, nitrogen is the most important for the production of drugs with high content of active principles. Only in a few cases is phosphorus and, still more seldom potassium, able to raise the content of active principles in a significant manner by overdosage.

5. If the active principle is a mixture of compounds (as is mostly the case) the total amount of the mixture is altered more than the proportion of the components of the mixture[28,59]. This means that these side reactions of metabolic processes, responsible for the production of the active principles, are affected as a whole rather than altered in themselves.

6. The amount of green plant material is more affected than the percentage of active principles[55], the variation between the highest and the lowest yield being up to more than 500 per cent.[55]. On the other hand, the variation of the active principles caused by factors of the soil is seldom higher than 40 per cent. and may only exceptionally become more than 100 per cent. In most of the cases the influence of the soil factor may be even lower than 20 per cent.

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