CONCLUSIONS

The experiments herein described lead to the following conclusions:

1. Different agronomic varieties of Cannabis vary markedly in chemical makeup of resin as evidenced by wide differences in response to the alkaline Beam test.

2. Treatment of the soil with various fertilizers alone and in combination is without effect on alkaline Beam test response under the conditions studied.

3. Male and female plants respond essentially alike to the alkaline Beam test.

4. Intensity of response to the alkaline Beam test tends to increase with age of plants at least until the time of flowering.

5. Intensity of response to the acid Beam test was not definitely influenced by varying variety, fertilizer, sex or age of plants except that there was observed a statistically significant diminution in intensity after the plants had flowered.

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Polyploidy in Relation to Chemical Analysis

*By Wm. J. Bonisteel**

Polyploidy is present in nearly one-half of the flowering plants. Economic plants that are polyploid in nature include apples, wheat, oats, corn, tomatoes, tobacco (1) and many other plants that are of horticultural interest. Polyploids are organisms that have more than two sets of homologous chromosomes. Cytological and genetical studies show that interspecific hybridization, chromosome duplication and meiotic aberrations have played an important part in the development of new and improved forms of plant life. The function that polyploidy exerts in the chemical constituents in the realm of drug plants seems to have been largely overlooked up to the present time. By the use of chemical substances it is possible to induce polyploidy in certain plants (2). Sterile triploids may have their chromosomes doubled and thus become fertile plants. Polyploid plants are generally characterized by larger size, vigor, hardiness and larger cells.

Polyploidy and Plant Constituents.—Plant breeding means the production and maintenance of new types through the direction and control of the natural processes of fertilization (3). Digitalis may be cited as a type that is carefully selected and bred for uniform constituents. That polyploidy induces chemical changes in the cellular constituents of plants has been shown by Crane and Zilva (1933) in their studies on the vitamin C content of tetraploid tomatoes which is approximately double that in the diploid plant from which they were derived. In the apple considerable variation is found in the vitamin C content between diploid and triploid varieties. Mangelsdorf and Fraps (4) have shown the effectiveness of genic changes in the production of the nutritional biochemical vitamin A when suitable pollinations are made in corn. Each gene for yellow induced approximately 2.5 units of vitamin A per Gm. of seed and they were able to increase the vitamin A content from 0.05 unit to as high as 5.00 units per Gm. Beasley (5) found that in tetraploid cottons doubling the chromosome number gave octoploids and the fiber length was increased. Such results show that a definite quantitative relationship exists between the genetic factors in a plant and the chemical substance produced.

Polyploidy and Toxicity in the Genus Aconitum.—In a previous paper (6) Sparks Aconite was investigated cytologically and this clone was found to have two somatic chromosome numbers. The majority of the root tips have the triploid ($3n = 24$) chromosome number but sectors were found in a few root tips which had the hexaploid ($6n = 48$) chromosome number. Thus Sparks Aconite is a member of the polyploid series of aconites. Preliminary tests indicate that this clone was toxic. It is to be noted that if a sector with 48 chromosomes reached a growing point a new type of plant would result. Since Sparks Aconite is a sterile clone and has never been known to produce seeds in nature or under adequately controlled hybridization pollinations such a 48 chromosomed plant would be fertile and produce viable seeds. Whether such a plant would be toxic or not is undetermined at the present time. Experiments now being conducted show that aconites can be induced to double their chromosomes by suitable treatment with colchicine. Treated plants show the characteristic stunted growth, thick heavy leaves and swellings indicative of chromosome doubling as a result of colchicine treatment.

A study was made of the chromosome numbers of several types of aconites and this was compared with the published chemical analysis and the assumed

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toxicity. It is necessary to recall that it is not always possible for the cytologist and the chemist to be dealing with the same type of plant. Cases are known where *Aconitum Fischeri* was used for what was supposed to be *A. napellus*. Nevertheless the tabulated results in Table I show considerable uniformity as the results indicate. When diploid plants are compared, it is generally found that the plants are non-toxic. Published results are not available for all the plants tested and in those cases preliminary tests for toxicity were made without a series of adequate controls. But these results compared favorably with those plants which had seemingly well-authenticated chemical analysis.

lished records. In a series of hybridization experiments over several years it has not been possible to duplicate this plant. Over 30 different plants have been grown that were procured as true *A. napellus* species, but when critically studied showed extreme variation. Many failed to set seed and pollen abortion was relatively high indicating a high degree of unstability as far as seed production is concerned. Many of these plants are clones and only by the careful examination of the type specimen is it at all possible to determine the present status of the many so-called *A. napellus* types. The type specimens (in Europe) are now buried so that they will be safe for the future.

Table I.—Polyploidy and Toxicity in Certain Species or Clones of *Aconitum*

Species or Clones	Chromosome Number	Type	Chemical Structure	Toxicity
A. heterophyllum	16	Diploid	$C_{22}H_{35}O_2N$	Non-toxic
A. paniculata	16	Diploid	$C_{29}H_{35}O_7N$	Non-toxic
A. variegatum	16	Diploid	....	Relatively non-toxic
A. noveboracense	16	Diploid	....	Relatively non-toxic
A. napellus	24 (?)	Triploid	$C_{34}H_{47}O_{11}N$	Very toxic
Sparks Aconite	24 Clone	Triploid	....	Considered toxic[a]
A. Stoerkianum	24 (?)	Triploid	.... (?)	Very toxic
A. napellus	32 (?)	Tetraploid	$C_{34}H_{47}O_{11}N$	Very toxic
A. chinense	32	Tetraploid	Aconitine (?)	Very toxic
....	(40)	Pentaploid	Not observed in nature	
Sparks Aconite[b]	(48)	Hexaploid	Not observed as yet	
A. palmatum	48	Hexaploid	Palmatisine (?)	Non-toxic
....	(56)	Septaploid	Not observed in nature	
A. Wilsoni	64	Octoploid	....	Undetermined
A. Delavaji	64	Octoploid	....	Undetermined

[a] Preliminary tests indicate toxicity.
[b] Hexaploid sectors in root tips if extended into growing points would produce a fertile plant from the sterile triploid clone.

The surprising fact is that the triploid and tetraploid aconites are very toxic. In this category is found aconitine. Some doubt exists as to the exact chemical composition of this alkaloid. Based upon the findings of cytology it may be noted that *A. napellus* seemingly has 24 chromosomes and also 32. Are these two different plants? This question can only be answered by an adequate cytological study. In the case of Sparks Aconite cytological studies show that the 24 chromosomes can be resolved into three sets. The parentage is not known. But from a critical analogy with other known plants it is assumed to be a hybrid arising from (1) two diploid plants in which one gamete was unreduced or (2) one of its parents could have been a tetraploid (double diploid) and the other a diploid. The problem is complicated since the parents of this clone are not known. On the assumption that part of its inheritance was diploid in origin is it to be assumed that the relatively non-toxic atisine type of aconites characterized by the diploids gave rise to a very toxic alkaloid? Some factorial change may result in the production of new molecules or transferring other radicals into new and more toxic positions. Does doubling of chromosomes confer toxicity on a relatively non-toxic plant? Based upon the data presented the evidence points in that direction.

A. Stoerkianum (given the status of a species) is evidently a hybrid between *A. variegatum* and *A. napellus*. The plant is toxic according to the pub-

Only one hexaploid aconite has been examined chemically and it is considered to be relatively non-toxic. The alkaloid is palmatisine from *A. palmatum*, a plant growing in India. It would be mere speculation to guess at the relationship of this plant. It could have arisen as a result of chromosome duplication of a triploid. The plant is known to set viable seeds. Induced polyploidy in Sparks Aconite would produce a hexaploid. Such a hexaploid form is now being synthesized by suitable chemical treatment. Its toxic action cannot be predicted but it will be interesting to see if doubling a triploid will result in reduced or increased toxic action. Based upon the evidence from *A. palmatum* such a plant would be relatively non-toxic.

Plants with 40 and 56 chromosomes have not been found in the aconites growing in the wild. It is possible, however, to synthesize them if such were desirable. It has been possible to produce nearly a hundred types of aconites from the available breeding stock at hand. These plants possess all the characteristics that are present in the polyploid plants.

The octoploid aconites have not been investigated and there is no criterion to guide us except the cytological findings. Preliminary tests for toxicity were unsuccessful in this group, and material available was not sufficient for further study.

Cinchona Alkaloids and Hybrids.—Useful published results on the cytology of the genus Cinchona are very meager but it is to be assumed that considerable work has been done and that it will never be published. Whether the Americas will awake to the need of this important plant-breeding problem in sufficient time to prevent a shortage of this drug is indeed questionable. The chemist will undoubtedly be able to make synthetic quinine but the product has not yet reached the flow sheet stage in industry. From a study of the Columbian cinchonas, the writer found that many of the types were of such a nature that under suitable breeding programs plants could be produced that would yield high alkaloidal products. It is to be recalled that the parents of all high-breeding cinchona stocks were derived from the native cinchonas of the Andean highlands. The data in Table II show the hybrid yield of two cinchona crosses. The first hybrid, which is a cross between *Cinchona succirubra* and *C. officinalis*, actually gave a lower percentage of quinine than either of the parents. Cinchonidine is increased over the yield in either parent. Cinchonine shows but little difference and the total yield of all alkaloids seems but little changed. It is obvious that such a cross is undesirable but it does indicate that some genetical factors are responsible for this condition.

of these hybrids it is possible to increase the toxicity of the aconites.

In the recent monograph published by the AMERICAN PHARMACEUTICAL ASSOCIATION on the genus Aconitum no mention was made of the chromosome number. Neither were any references made to the cytology of this group which clearly pointed out many irregularities which must be taken into consideration in this group with so many differing alkaloidal complexes. Many brilliantly executed papers have been published on the various phases of the aconite problem but one is never certain of the exact aconite that was used in the experiment.

Colchicine and Induced Polyploidy.—The alkaloid colchicine has the remarkable property of inducing polyploidy in certain plants. Seeds, buds and other parts of the plant are treated with water solutions with from 0.05 per cent of colchicine to as high as one per cent for varying periods of time. Solutions may be prepared in agar or in lanolin mixtures. The details of this procedure are adequately covered in the literature (2) and need not be repeated.

In the experimental work with the aconites the plant is extremely stunted by colchicine and shows only a few leaves while controls will often reach a

Table II.—Results of Alkaloidal Yield in Hybrid Cinchonas *vs.* Parents. Only Three Important Alkaloids Considered

Species or Hybrids	Quinine, %	Cinchonidine, %	Cinchonine, %	Undet., %	Total Alk., %
C. succirubra	1.91	1.14	2.11	0.85	6.04
C. officinalis	2.77	0.39	1.57	0.66	5.39
Hybrid of above parents	1.43	1.58	1.58	0.81	5.40
C. succirubra[a]	2.54	2.05	2.14	1.59	8.32
C. Ledgeriana[a]	3.81	0.00	0.04	1.15	5.00
Hybrid of above parents	6.77	0.73	..	..	11.83

[a] Cultivated species.

The cross between *C. succirubra* (7) and *C. Ledgeriana* gave a hybrid whose yield of quinine was nearly 100 per cent higher than either parent. These plants were cultivated and naturally did not have to compete with other plants for food. This is an excellent example of the marked heterosis that is often shown in hybrids. The low percentage of cinchonidine present in the hybrid shows that some factor in the chromosome complex shifted part of this molecule toward the quinine group. Since factors are grouped according to their most conspicuous effects it is proposed that the genes which exist in this hybrid should be called the genes intensifying quinine. The total yield of alkaloids in this hybrid is high but it is known that much higher yields have been produced.

Similar results have been obtained in the breeding experiments with the genus Aconitum. Many hundreds of crosses tried have been failures. Many of the failures have been due to abnormal meiotic behavior and as such do not fall within the scope of this paper. On the other hand some successful crosses have proved to be worthless plants. Still other plants have excelled the parents in the vigor of the stock, period of flowering and hardiness. Preliminary tests for toxicity indicate that in many

height of 0.5 meter. The leaves of the treated plants are variously deformed and have a thick leathery appearance. Many of the plants die but this has been the fault of the experiment in using solutions that were too strong. It is necessary to vary the solution with each type of plant. The pollen is also an index of the induced polyploidy for the successfully treated plants have larger pollen grains. While certain aconites respond to the action of colchicine others do not respond to any normal concentration.

Colchicine and Double Diploids.—The simple explanation of the action of colchicine upon the cell may be inferred from treating a diploid aconite species with its normal complement of 16 chromosomes. In ordinary cell division there would be in the somatic tissues two cells each with 16 chromosomes. In the colchicine-treated plants the chromosomes are doubled since the mechanism for the separation of the chromosomes is arrested and the resulting cell has 32 chromosomes. This is a tetraploid plant or, in terms of the newer terminology, a double diploid.

In the cytological investigations with Sparks Aconite cells were found with 24 chromosomes in the root tip. In Fig. 1 the somatic chromosomes of this triploid are illustrated.

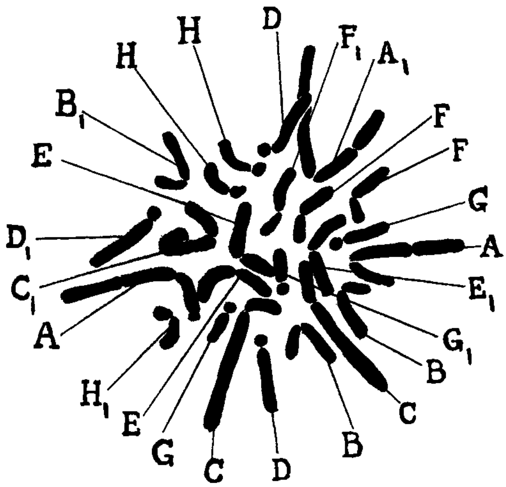

Fig. 1.—Somatic Chromosomes from the Root Tip of Sparks Aconite. Fixed in La Cour's 2BD and Stained in Newton's Iodine Gentian Violet ($3n = 24$).

In Fig. 2 the semi-diagrammatic sketch shows the relative size and shape of the three sets of chromosomes that are found in the root tips of Sparks Aconite. The detailed description of the size, shape and constrictions present in the three sets of chromosomes is omitted in this paper. However such information is necessary for any cytological investigation.

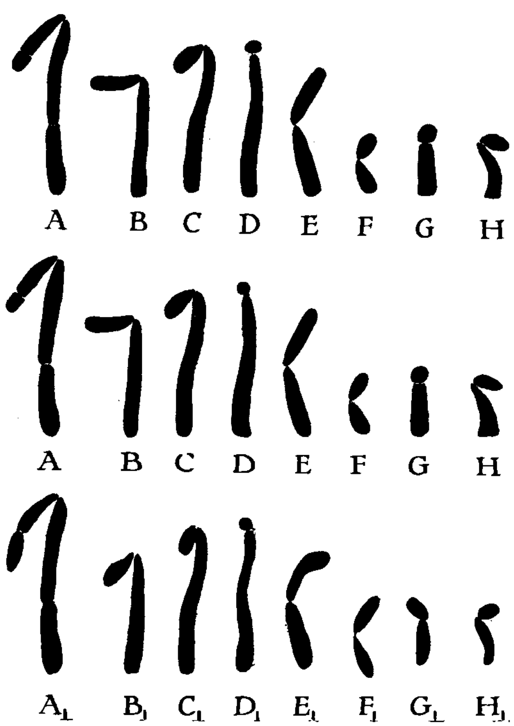

Fig. 2.—Semi-diagrammatic Drawings Showing the Relative Size, Shape and Constrictions Present in the Chromosomes from the Root Tips of Sparks Aconite. The Two A Sets Are Alike While the A_1 Set Differs in Length of Arms, Extended Knob at Tips and in Compactness.

In Fig. 3 the chromosomes found in the somatic cells with the hexaploid number of chromosomes is shown. It is to be noted that in the diploid three sets of chromosomes are designated A, A and A_1, etc. In the hexaploid these chromosomes are found doubled, that is, they are AA, AA, AA, AA, A_1, A_1, A_1, A_1, etc. This is exactly what happens when colchicine is effective in causing chromosome duplication.

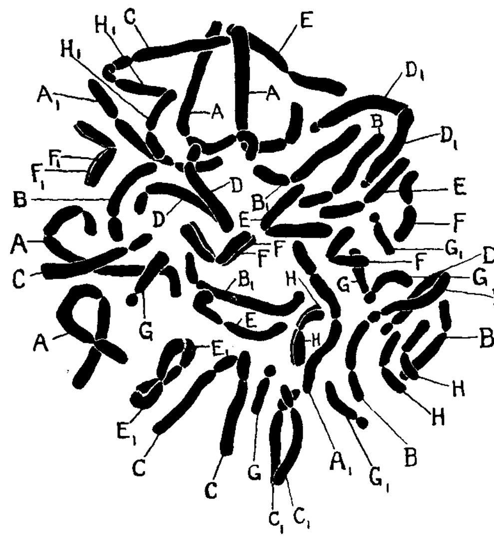

Fig. 3.—Somatic Chromosomes in a Single Cell from a Sector in the Root Tip of Sparks Aconite Which Have the Hexaploid ($6n = 48$) Number of Chromosomes. Cytological Technique as Indicated in Fig. 1. The Chromosome Duplication in This Clone Is Exactly What Takes Place When Colchicine Induces Polyploidy in Certain Plants.

In the plant world doubling of the chromosome number has occurred rather infrequently but one might have to wait a lifetime for this to occur. In Sparks Aconite this was found under experimental conditions as a normally occurring phenomenon. As has been indicated a growing point of this plant with a chromosome sector of 48 chromosomes could give rise to a new type of plant. Based upon other factors this plant would be fertile while the original triploid is sterile. Experiments to synthesize this hexaploid plant by means of colchicine treatment are now in progress.

SUMMARY AND DISCUSSION

In the genus Aconitum toxicity of the plants varies with the chromosome number. Diploids are usually non-toxic while the triploids and tetraploids represent the extremely toxic alkaloids of the genus. Hexaploids are considered non-toxic and no definite information is available about the octoploid forms. All the forms discussed are normally found growing wild with the exception of the triploids and these are often found wild as escapes from gardens.

Cytological evidence indicates that many of the triploids are plants in which diploids may have been the parents. It is to be assumed that many of these plants arose through chromosome duplication in cases of incompleted mitosis. Hexaploid sectors in the root tips of Sparks Aconite are clearly cases in which chromosome duplication might lead to the establishment of a new race.

With chemicals that induce polyploidy the plant breeder is provided with a tool that may enable him to produce double diploids or to treat sterile triploids so they may set seed. This is one of the fertile fields of research for the breeder of drug plants. The plants produce chemical substances with greater ease than the chemical laboratory.

Chemical analysis of cinchona hybrids indicates that a shift from one alkaloid to another may take place in the hybrids that have been produced. Such changes are known to occur in other plant products. In this, as yet unexplored, field some fundamental answer must be sought to explain the change that takes place in the chromosome mechanism which underlies these shifts in radical position or that exerts so profound an effect as shown in toxicity. It is suggested that the chromosome number be considered in the chemical analysis of these plants with a polyploid series of chromosome numbers. The status of the plant should be carefully considered. This is imperative with the problem of induced polyploidy through chemical treatment of plants. In the future all monographs published by the AMERICAN PHARMACEUTICAL ASSOCIATION should consider the cytological problems that are encountered in the preparation of monographs of drug plants.

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A Brief History of Commercial Medicinal Plant Culture in the United States*

By A. F. Sievers†

The desire to grow medicinal plants for the market comes to people of many classes, occupations and circumstances. Farmers, urban workers and business men at times become interested in such an undertaking, as evidenced by the hundreds of letters received annually by the U. S. Department of Agriculture requesting information on the subject. In most cases these inquiries are prompted by the belief that medicinal plant culture is a new and profitable enterprise. The high cost of medicines naturally suggests to many people that the plants furnishing the products that go into such medicines must be of high market value. Quite the contrary is the case. Botanical drugs, on the whole, are quite cheap and therein lies the principal reason why medicinal plant culture is not a well-established agricultural industry in this country. With few exceptions, our requirements of the crude drugs come from one of two sources—they are imported from abroad or they are collected from domestic wild plants. Many come from the tropics where they are grown or collected with very cheap labor.

Interest in the subject is usually greatest in periods when persons are more or less dissatisfied with their lot or forced to seek new means of a livelihood. During depres-

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