THE POSSIBILITY OF INCREASING THE ALKALOIDAL CONTENT OF BELLADONNA PLANTS THROUGH SELECTION.*

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For a good many years importers and jobbers of drugs have experienced difficulty in supplying certain drugs of the required strength. Even previous to 1900, the belladonna and stramonium which could be secured was alleged to be of such inferior quality that it was impossible to furnish sufficient quantities of these drugs of the Pharmacopœia Standard. This difficulty was finally recognized by the revision committee of the eighth decennial revision of the Pharmacopœia, and the required strength of each of these drugs reduced by .05 percent of alkaloids.

Whatever may have been the wisdom of making this reduction in the standards, the fact that it was made first called serious attention to the apparently growing inferiority of some of the important official vegetable drugs, a situation which was viewed with considerable alarm, for if any further deterioration should take place in the future, as seemed very likely, it would become necessary in time to still further reduce the standards with the consequence of bringing a lot of inferior material on the market. The rapidly diminishing supply of belladonna from the wild state makes its cultivation in the near future practically a necessity and since cultivation of a naturally wild plant often results in decreasing its strength, it seemed highly desirable that something be done to improve the quality of this plant.

While the Solanaceous group of plants are not by any means the only ones which could be improved to advantage, the Office of Drug Plant Investigations in taking up this problem selected this group on which to begin the study of medicinal plant improvement for several reasons. In belladonna and hyoscyamus the group includes two of probably the most important and most widely used vegetable drugs. Two of the members of this group were largely responsible for the attempt to investigate this question in that the widespread inferiority of the official material they furnished necessitated the lowering of the Pharmacopœia standard, thus calling attention to a question of grave importance. The active principles which they contain being definite chemical compounds which admit of definite quantitative determinations the plants lend themselves well toward studying individuals. Finally, belladonna, being a perennial, can be studied through a number of successive seasons which is a further distinct advantage.

The investigation was started on its present scale in the spring of 1911. The first step was entirely in the direction of studying a large number of individuals to determine the following principal points: (1) Is there a deterioration in the belladonna plant under cultivation, in so far as its medicinal quality is concerned? (2) If such is the case is the deterioration general of the whole plant or is it found only among certain individuals? (3) Does this individual characteristic

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manifest itself at different stages during the growing season? (4) Is there any relationship between the medicinal value of the plant and its physical appearance or in other words, its vigor, growth, and general thriftiness? (5) Is a high alkaloidal content in an individual plant of perennial occurrence or does it only occur in one season?

The season of 1911 was started with 59 individual plants under observation. Each plant was carefully watched as to growth and appearance with a view towards finding some striking physical characteristic which might later serve as a type. Samples of the leaves were collected at the following stages of growth: First stage, during the early part of May before the flowers appeared; second stage, during the latter part of May when the plants were in full bloom; third stage, about the middle of June when the berries were developing in various stages of maturity; fourth stage, early in September when the berries were mostly ripe; fifth stage, about the middle of October after the plants had made considerable growth of new leaves. Practically the same plan was followed in 1912 with the same list of plants.

The results of the two years' investigation show two interesting facts. Taken as a whole, the plants under cultivation at Arlington for these experiments show a comparatively high alkaloidal content in their leaves. Of the 59 plants in the list, not a single one fell below the present standard of .3 percent in its season average for either year and only one assayed below the old standard of .35 percent. This would seem to show that in this particular location at least, belladonna plants can be grown under cultivation which may be expected to yield a product considerably above the standard required at present.

The other striking fact brought out is the wide range of variation in the alkaloidal content of the individuals. Table I shows the maximum and minimum percentage found at each stage of growth among the 59 plants at Arlington, 10 plants at Madison, Wisconsin, and 19 plants at Bell, Maryland.

TABLE I.

Range of Variation in Percentage of Alkaloids in Leaves.

Stage of Growth	Arlington, Va.				Madison, Wis.				Bell, Md.	
	1911		1912		1911		1912		1911	
	High	Low	High	Low	High	Low	High	Low	High	Low
First	0.852	0.303	0.869	0.404	0.580	0.418	0.500	0.268	0.823	0.329
Second	.879	.267	.747	.292	.820	.427	.519	.316	.783	.288
Third	.925	.277	.882	.328	.767	.419	......	......	.750	.350
Fourth	.908	.311	.806	.359	......	......	......	......	......	......
Fifth	.733	.200	.678	.296	......	......	......	......	......	......
Season's average	.766	.306	.768	.353	.665	.430	.452	.312	.707	.346
Average	.841	.277	.792	.339	.708	.423	.490	.298	.766	.339

Alkaloidal Content at Different Periods of Growth.—There being not sufficient time to show on the tables the alkaloidal content of all the plants at all the stages of growth, a few of the typically rich and poor plants have been selected and the complete data shown in Table II.

TABLE II.

Plants with Leaves of Low Alkaloidal Content.

Stage of Growth.	No. 3 1911	No. 3 1912	No. 23 1911	No. 23 1912	No. 34 1911	No. 34 1912	No. 46 1911	No. 46 1912
First	.384	....	....	.496	.335	....	.337	.418
Second	.375	.393	.348	.366	....	.292	.285	.334
Third	.277	.448	.354	.341	.526	.320	.308	.480
Fourth	.549	.448	.487	....	.532	....	.588	.483
Fifth	.451	....	.425	....	.200	....	.431	.314
Average	.407	.429	.403	.401	.414	.406	.390	.406

Plants with Leaves of High Alkaloidal Content.

Stage of Growth	No. 21 1911	No. 21 1912	No. 29 1911	No. 29 1912	No. 1W 1911	No. 1W 1912	No. 6W 1911	No. 6W 1912	No. 7W 1911	No. 7W 1912
First	....	.732	....	.737	.638	.737	.596	.847	.558	.782
Second	.535	.719	.655	.647	.835	.642	.879	.747	.831	.666
Third	.633	.781	.914	.729	.587	.777	.925	.882	.832	.646
Fourth	.669	....	.908	....	.738	....	.711	.804	.727	.694
Fifth	.684	....	.547	....	.612	....	.722	.558	.571	.573
Average	.630	.744	.756	.704	.682	.719	.766	.768	.704	.672

Alkaloidal Content During Successive Seasons.—It having been fairly well established that the characteristic of a high or low alkaloidal content in certain individuals manifests itself during the entire growing season we come to the question of the perennial consistency of such a characteristic. This is of prime importance for the possibility of improvement by selection depends on it. If it is found that a plant selected for its high content of alkaloids one year is only of ordinary quality the following season, it would seem likely that seasonal influences are mostly responsible for the relative development of alkaloids and that such a property could not be used to advantage as a basis for selection. If, on the other hand, it is found that the high alkaloidal characteristic exists in the same plant through a number of seasons it is logical to assume that the property of forming large quantities of those alkaloids is an inherent characteristic in that particular individual plant in distinction from the others grown under similar conditions.

In Table II in which the analyses of typical plants are shown for two seasons, we find that these plants display the same characteristic throughout both seasons. Numerous other plants can be selected from the list under observation. It would seem that in plants which are conspicuously rich or poor in alkaloids, as numbers 6w and 7w and numbers 3 and 34, the tendency to produce such large or small quantities of alkaloids constitutes a definite characteristic which is manifested by that individual plant throughout its existence in much the same way as certain types of sugar beets produce a conspicuously high or low precent of sugar.

Relation of Physical and Chemical Characteristics.—As previously stated, the plants under investigation were carefully noted as to their physical appearance with a view to finding some relationship between that and the alkaloidal content. Thus far, nothing has been found to indicate that such a relationship exists.

The belladonna plant is almost entirely lacking in any distinctive physical features, any one of which might serve as a distinct type. While the size of each plant, the number of stems it has and its general thriftiness have been noted, nothing has been found which is characteristic of any one individual. It would be a distinct advantage if plants of a desirable alkaloidal content could be identified by means of some distinctive physical feature since that would make the tedious assay process unnecessary in many cases.

Method and Results of Selection.—It having been established that the variation in the alkaloidal content of belladonna plants can possibly be made the basis for the improvement of the plant, the next step in the problem is to apply the methods of selection. The belladonna plant may be propagated by seed or by cuttings. The flowers of the plant are well adapted to insect fertilization and in order to insure close pollination, bagging or screening is necessary. In selecting the plants to be used, types of very good, very poor, and average alkaloid producing individuals were chosen. While the plants of poor alkaloidal content are of no economic significance they were included so as to give more extensive data on the possibility of reproducing in successive generations the characteristics regarding alkaloid production.

In 1911, the attempt to secure close-pollinated seed was not successful. Open pollinated seed from the selected individuals were sown in the greenhouse in January and in April the seedlings were set out in the plat. The plants made a slow but steady growth and on the 23d of July, when the plants were in full bloom, the first picking was made. The number of plants secured from each selected parent varied from six to fifteen. No individual picking was made, but a general representative sample of leaves was secured from all the plants from each selected individual. On August 30th, when the berries were partially ripe, a second picking was secured in the same way. Of the five plants of high alkaloidal content shown in Table II, numbers 21 and 29 died of a root disease before seeds could be secured and number 1w was in such a diseased condition that the seeds which were secured failed to germinate. Numbers 6w and 7w and also numbers 12 and 13, each rich in alkaloids, yielded first generation plants from their seeds. Plants were also secured from the seed of numbers 3, 34 and 19, all plants of low alkaloidal content. Table III shows the results of the assays.

TABLE III.

Percentage of Alkaloids in the Leaves of First Generation Plants Grown from Seed from Selected Individual Plants.

	Percent of Alkaloids, Parent	First Stage	Second Stage	Average
3	Low	.524	.693	.609
34	Low	.479	.518	.498
19	Low	.493	.519	.506
12	High	.650	.882	.766
13	High	.640	.859	.750
7w	High	.617	1.063	.840
6w	High	.805	1.282	1.043

Attention is directed here to the striking superiority of plants 6w and 7w over the others. Leaves from the second picking of 6w contained a percentage of alkaloids probably never met with previously in belladonna.

During the past summer, these plants have all been picked individually and while only a few of the samples have been assayed, the results so far indicate quite clearly that the individuals from 6w will average much higher than any of the others. This would go further to show that the individual plant 6w which shows to such advantage through 1911 and 1912 and whose first generation reproductions rank highest collectively the first season will serve as a good type to form the basis of further propagations. During 1912, close pollinated seeds were secured from this and several other good types and the plants from these seeds are now making their first year's growth and are being examined and tested individually. The parent plant 6w in now dead, but it is hoped that some of its reproductions will continue to prove equally good if not better. This fall cuttings are being made from the most desirable plants and it is believed that this method will prove the quickest and most productive means of propagation.

Some General Considerations.—The problem under discussion, like most problems in plant breeding and selection, must extend over a good many years. What has been accomplished thus far constitutes practically merely a beginning because we have not yet embarked on any breeding operations, but have only supplied the information and material necessary to make the application of the proposed methods possible. However, while in the actual solution of the problem only a step has been made, the future work will likely entail much less actual labor in that the many analyses which were necessary in the earlier phases of the work can be largely dispensed with and the investigation limited to a few selected plants.

It has no doubt occurred to you that the increase of alkaloids in the plant will in itself alone not relieve the economic situation. From what has been said of the relationship of the size and thriftiness of the plant, there exists the possibility that the plant richest in alkaloids may also prove to be a poor type from an agricultural standpoint. The ideal plant must, therefore, combine chemical and physical excellence in order to constitute the best plant from both the therapeutic and agricultural standpoint.

Much has been said and written regarding the influence of soil and climate on the production of alkaloids and the conclusions reached have varied greatly. In view of the great variation existing in individual plants as shown here, grown under identical conditions as regards soil and climate, it must be plain that little if any importance can be attached to experiments designed to show the influence of these factors until a type of plant can be secured which shows the minimum amount of individual variation. Numerous instances are found in literature where differences in the alkaloidal content of belladonna plants which are claimed to be the results of fertilizers or climatic conditions are considerably less than variations found in the fifty-nine plants here studied. There is no doubt that environmental factors have some influence but the extent of such influences can not be studied under present conditions with any degree of certainty.

This investigation has offered us a splendid opportunity to study the belladonna plant itself. Time will not permit the discussion in any detail of the various points of interest that have been worked out in this connection. Suffice it to say that we have found a practical way to grow belladonna from seed that eliminates the difficulties of field sowing. The various details regarding the germination of the seed have been worked out. Furthermore, the abundant material on hand made it possible to study thoroughly the distribution of the alkaloids in the various parts of the individual plant; the development of alkaloids in the seedlings and early stages of growth and the relative concentration of alkaloids in the leaves with relation to age and size.

As has already been said, belladonna was chosen for this work for certain reasons. If the problem is finally brought to a successful conclusion its value will lie not so much in what has actually been done with one plant as in the fact that it points the way to the possibility of a broader application of similar methods to our field of medicinal plants.

SENSITIZED VACCINES.*

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Sensitized Vaccines, Sero-Vaccines, or Sero-Bacterins, are suspensions of pathogenic bacteria, living or dead, artificially sensitized by treating them with immune homologous serums, i. e., serums from animals immunized against bacteria of the same kind as those used for producing the Vaccines. By this means the amboceptors contained in the immune serums are made to combine with the bacteria and sensitize them, so when they are injected into the body the complement and phagocytes normally present in the blood of the injected individual immediately combine with and digest them, and the resultant products stimulate the tissue cells to produce antibodies to which the subsequent immunity resulting from the vaccination by the sensitized vaccine is due.

How the Immune Serum is Obtained.—The immune serum for making sensitized vaccines is usually prepared by treating goats intravenously, first, with dead, and later with living cultures of bacteria. Trial bleedings are made at regular intervals, and the serum is tested for amboceptors and other specific antibodies. When the serum shows a sufficiently high titre, a large quantity of blood is withdrawn for use in preparing the sensitized vaccine.

How Vaccines are Sensitized.—The bacterial cultures to be sensitized are added to a little physiological salt solution, emulsified, turned into a vessel containing the immune serum, allowed to macerate for a few hours, the clear and slightly opalescent liquid separated from the deposit of bacteria, and the latter washed by centrifugalization several times in physiological salt solution until the last traces of serum disappear. The white mass thus obtained is of a pasty, semiliquid consistency, and after standardization by bacterial count, and the addition of physiological salt solution in proper amounts, produces an entirely homogenous emulsion which constitutes the sensitized vaccine.

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