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ISOTOPES IN AGRICULTURE

A Monograph By

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ABOUT THIS MONOGRAPH: A review is presented of work in the U. S. A. on radioinduced mutations in plants and animals, fertilizer tracer studies, development of smut and rust-resistant plants, and animal metabolism and tissue distribution tracer studies.

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With the establishment of the Atomic Energy Act in 1946, Congress wisely decided that provision should be made to encourage work in fields that would prove of benefit to mankind. Accordingly several divisions within the Atomic Energy Commission initiated such programs. One of these, the Division of Biology and Medicine, established broad programs in medical and biological studies.

I mention the background of this Division of the Atomic Energy Commission because too many people think of the Commission only in terms of bombs and instruments of war quite overlooking the peacetime accomplishments. Actually the Commission has been the single most important force in applying nuclear energy and its products to biological and agricultural problems. The record is given in the semi-annual report to Congress for the period July-December 1954. The Biology Branch alone lists 195 titles of research projects being carried out at Colleges, Universities and foundations in practically every State in the Union. In the broadest interpretation, every one of these studies is related to some phase of agriculture. This type of work is spoken of as off-site research and is supported jointly by the Commission and the participating institution. It is work which is proposed by the worker at the institution and although of interest to the Commission it is probably of greater interest to the institution. Even so the Commission bears a large part of the expense of the study.

In addition to this program, there is a large volume of research of both direct and indirect interest to agriculture which is spoken of as on-site research and is conducted at the National Laboratories. This work is completely supported by the Commission because it is of great value to the Commission's program.

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*Miscellaneous Paper No. 11, Oregon Agricultural Experiment Station.

One of the most important and spectacular studies is being conducted by Dr. Ralph Singleton of Brookhaven National Laboratory. He has established what is known as a "Gamma Field" consisting of a large securely fenced plot which has in the middle of the field a 2000 curie source of Co^{60} which can be raised or lowered by remote control. Various plants are placed in concentric rings around this source so that the amount of radiation can be controlled ranging from a few curies per day to large amounts. Many species have been studied and it has been found that the response is greatly different with the different species with some being quite resistant while others are very sensitive to irradiation.

We know that the characteristics of any organism are determined by the genes present in the chromosomes. If they are exactly the same as the parents the offspring are exact duplicates of the parents. However if a mutation, a change in the genes, occurs there will be a difference in the offspring.

There are many ways to cause mutations but one of the easiest and most economical is by the use of ionizing radiation. Dr. Singleton is a geneticist who has worked on corn genetics for many years so it is not surprising that this species has received special attention. He has produced variations all the way from short stocky plants to tall slender varieties and in some cases has produced what looks to the untrained observer to be grass. As you probably know, corn is actually a grass so it has returned to the form from which it originally came.

Most mutations are bad but occasionally a good one does occur and this is the only way we can hope to get new varieties. Therefore any condition that speeds up the mutation rate also increases the chance of finding a promising new variety. This of course is the reason for using the Co^{60}.

In one experiment on corn, Dr. Singleton reported that after examining 25,150 kernels of corn from non-irradiated plants, he found 28 mutations. After irradiation with 1320 R, one time only, examination of 1000 kernels yielded 110 mutations. That is without irradiation there was approximately 1 mutation per 900 kernels occurring spontaneously while after irradiation there was 1 mutation occurring for every 9 kernels. Another way to say the same thing is that the plant breeder could accomplish in one year what would otherwise take 100 years.

Working with eight Universities and Agricultural Experiment Stations a program is underway where large numbers of fruit trees, shrubs and vines and even commercially valuable forest trees are growing being continuously exposed to ionizing radiation. Abnormal shoots and branches are occurring and when they do they will be cut off and grafted to normal stocks and the undesirable mutations will be screened from the desirable ones. An example is this carnation with one white and one red flower coming from the same parent stock.

Another phase of this program is the work of Dr. Gregory of North

Carolina State College. Using peanuts he was able to develop varieties which produced 30% higher yield per acre. Other varieties proved very resistant to the leaf spot disease.

Dr. Konzak's Brookhaven studies with oats is still another example of the application of ionizing radiation to plant breeding. The object was to develop a rust resistant high yielding variety of oats. By optimistic standards this would require ten years. After $1\frac{1}{2}$ years Dr. Konzak has a variety which meets these specifications, an extremely remarkable accomplishment.

Another important use of Atomic Energy products is the application of fertilizers. With some crops it was noted that phosphate fertilizers might give a response at the first part of the growing season which seemed to diminish as the plant matured. Was the phosphate actually fertilizing the plant during the whole growing season or was some of the value lost? To answer this question radio-phosphorus was carefully placed as to depth and distance from the plant. The plant was grown and harvested after various time intervals and analyzed for P^{32}.

With some varieties, particularly corn, it was found that the feeding root system was below the usual zone of phosphate placement and as soon as the plant was beginning to mature it no longer was getting the benefit of the applied phosphorus. To make use of this method of study a large scale experiment was begun under the joint sponsorship of the Atomic Energy Commission and the United States Department of Agriculture. A central mixing plant was established at Beltsville and the labeled phosphorus was mixed in the proper proportion. Twenty states, two territories and one foreign country participated in these studies. Many factors were studied such as root zone of the various crops, time of application of the phosphorus, effect of temperature, effect of moisture and effect of soil types on the phosphorus uptake. These findings have been most useful and with some crops have resulted in substantial savings.

Another phase of the fertilizer work has been reported by Dr. Turkey from Michigan State College. He has studied the foliar absorption of a number of nutrients and has shown that in some cases at least foliar absorption is much faster and more efficient than absorption through the roots. He has calculated the leaf surface of a 12 year old apple tree to be $\frac{1}{10}$ of an acre, while the tree occupies only $\frac{1}{100}$ of an acre. Thus it is not surprising that this route is a very efficient way of supplying water soluble nutrients.

Dr. Stakman, the famous plant pathologist has used ionizing radiation to cause mutations to occur in smuts and rusts. He has stated that it is possible that a whole cereal crop could be destroyed in a single year if the mutation of the rust caused a very virulent mutation to appear. What Dr. Stakman is doing is to speed up the mutation rate by irradiation so that the virulence will appear and then to produce a variety of the cereal which will be resistant to the virulent organism. The history of wheat culture has shown that no variety of

wheat can stay resistant for more than a few years. During the last forty years four distinct periods occurred where large losses were experienced. Therefore it is of the greatest importance to know what could occur and to have resistant varieties ready when these new races appear.

The effect of ionizing radiation on animals is just as real as is the effect on plants. It is perhaps not as dramatic and certainly is not as easily studied as with plants, as large numbers of animals must be used. I might briefly refer to the large mice experiment under the direction of Dr. Russell at Oak Ridge. By using easily recognized mutations he has been able to show the mutation rate is markedly speeded up. The possible application in these findings is less obvious than with plants, although it may in time prove as valuable.

There is a large number of experiments in the field of agriculture which use the isotopes as tracers in both plants and animals. The most useful isotopes are C^{14}, P^{32}, S^{35}, and I^{131}, although this by no means begins to cover all the important ones.

A study of the mode of action of 2,4-dichlorophenoxyacetic acid, commonly known as 2,4-D, the weed killer, is typical of these studies. Although we do not have all the answers we do know a great deal about this compound and how it behaves in the plant. Several syntheses have been carried out with the C^{14} in different positions thus allowing the investigator to follow the radioactivity through the plant. The radioactive compounds can be located by the use of counting equipment, separated and identified by the use of paper chromatography.

In addition to marking the individual atoms the tracer technique has the advantage of ultra sensitivity. It has been calculated that 1 milligram of C^{14}, which would give 200,000,000 disintegrations per second, and if synthesized into a compound and given to a 1,000 pound cow could be detected in a 10 milligram sample of blood, milk or tissue. At five centers the metabolism of dairy cattle as well as other economically valuable animals is being studied by the aid of isotopes. Let me quote some specific examples.

Since calcium and phosphorus may be re-excreted into the gut it is very difficult to determine the true availability of these elements in a foodstuff. By using isotopic forms it becomes a problem which can be easily answered. The isotopes are injected into the blood stream and if activity is found in the intestinal contents it could only come from material being re-excreted into the gut. For example it was shown by California workers that the apparent digestibility of phosphorus in alfalfa was only 22%. By using isotopic techniques the true digestibility was shown to be 91% thereby proving that alfalfa was a good source of phosphorus.

The recent observation of Dr. Comar that molybdenum is an essential element for animals is another case where isotopes proved invaluable. The use of Co^{60} in the rumen synthesis of vitamin B_{12} is still another example. If the Co is given parentally it is not nearly so effective.

Many workers believe the adaptability of various breeds of cattle to high temperatures and humidity may be directly related to thyroid function. This question is being studied by the use of I^{131} since the uptake of iodine is easily followed by in vivo studies.

It has been known for some time that ruminants could synthesize the sulfur containing amino acids, cystine and methionine from inorganic sulfur but it was quite surprising to learn that the chick could synthesize cystine from inorganic sulfur. When the chicks were fed S^{35}, the cystine was deposited in the protein of the feather. The radioautograph showed that the new protein was deposited at the base of the feather and the keratin already formed was not in equilibrium with body stores. Thus inorganic sulfur must be considered as an important and cheap nutritional element even for a non-ruminant.

The precursors of milk constituents are readily determined by ejecting the labeled substrate and examining the milk for C^{14} content. Thus acetic, Propionic, and butyric acids which we know are produced in large quantities in rumen fermentation are easily shown to be important substances in milk fat production. C^{14} labeled glucose is rather efficiently changed to milk sugar as well as other milk constituents.

These are only some examples of the application of isotopes to agriculture, but I believe they will give you some idea of the magnitude of this field and the possibilities inherent.