in acetone, but this leads to a change in relation of acetone to reagent.

(3) The hydrolysate in solution may be separated into fractions by the stepwise introduction of hydrogen chloride, either directly as the gas or by means of a strong solution of hydrogen chloride in acetone.

(4) Fractionation of solutions can be brought about by successive additions to the acetone-reagent-amino acid system of suitable amounts of some solvent which is miscible with acetone but which does not have a solvent action on the amino acid-reagent complexes.

(5) Trichloroacetic acid solutions of amino acids in acetone undergo spontaneous precipitation owing to the fact that amino acids catalytically decompose trichloroacetic acid into chloroform and carbon dioxide. As the acid is decomposed, the amino acids progressively precipitate. Chromatographic analysis of fractions obtained in this way show that individual amino acids do not drop out successively but that several come out together. There is, however, in this procedure a method for obtaining greatly simplified amino acid mixtures as compared with the hydrolysate from which the fractions are obtained.

Fractions of amino acids obtained by the above-described principles from hydrolysates of proteins have been submitted to examination by paper chromatography according to the method described by Consden and his associates (*1*). The results show that from hydrolysates containing 18 or more amino acids the components of a series of 20–60 successive fractions obtained by method 1 differ in their qualitative composition. Those amino acids most readily soluble in the reagent employed tend to be most abundant in the earlier, and the least soluble ones tend to accumulate in the later, fractions.

When such fractions are rechromatographed at successive dilutions, one after another of the constituent amino acids fails to appear on the developed chromatogram. By this means it is possible to secure an approximate appraisal of the quantitative composition of any fraction with respect to its component amino acids. Each amino acid in a fraction can be identified by chromatographing the fraction in parallel with seedings of pure amino acids. The known keeps step with the unknown and reveals its identity.

Application of the available color reactions for individual amino acids to the fractions obtained by the methods described reveals that these amino acids are not present throughout the series but are found only over limited ranges of the successive fractions.

Extensive fractionation of a hydrolysate, identification and approximate quantitative analysis of successive fractions, provide information as to the similarity of fractions. This may then be used as a basis for the recombination of similar fractions in order to reduce the number of fractions to be subsequently worked with in further separation of the components. In the case of certain fractions it has been found that refractionation with the same reagent may lead to further simplification of the composition of the mixtures of amino acids which they contain. But, as will be seen from the accompanying table, advantageous procedures for isolation of individual amino acids are available by changing to a second reagent for further fractionation.

We are pursuing our investigation of the possibilities of applying the principles here described to the isolation of individual amino acids from partially and completely hydrolyzed preparations from proteins. Our studies, details of which will form the subjects of later communications, have indicated the practicability of these methods.

Reference

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The Control of Grass Weeds in Sugar-Cane Fields in Puerto Rico

J. A. B. Nolla

Mayaguez, Puerto Rico

In the course of experiments and field tests conducted during the last three years with herbicides for the control of weeds infesting sugar-cane lands in Puerto Rico it was soon found that the grass weed population increased when the broad-leaved plants like "cohitre" (*Commelina logicaulis* Jacq.), *Amaranthus spinosus* L., and "bejuco de puerco" (*Ipomoea* spp.) were destroyed by 2,4-D or other herbicides. The need arose for an effective grass herbicide complementary to 2,4-D or for a substitute which might control both broad-leaved and grass weeds.

It has been generally recognized that 2,4-D has little, if any, effect on grasses. Recently, Mangual (*2*) reported that the addition of 2,4-D increased the herbicidal action of oil emulsion (pentachlorophenol in diesel oil) and Concentrate 40 in grass control. Crafts (*1*) recommends an oil emulsion contact spray (pentachlorophenol in aromatic oil) for the control of young grass seedlings and claims that the addition of 2,4-D to the spray further adds to its value, providing a lethal agent for the weeds easily controlled by 2,4-D.

The results of experiments conducted on various soil types with ample replications agree with the above on the value of pentachlorophenol and the combined 2,4-D-pentachlorophenol in the control of grasses. We have further found that 2,4-D in oil provides a more effective grass herbicide than pentachlorophenol emulsion or Concentrate 40.

In a typical experiment on fallow ground and repeated three times, plots were treated with an aqueous solution of 0.1% 2.4-D to kill most of the broad-leaved plants, allowing the grass weeds to develop without competition. The most prevalent volunteer weeds on the plots were *Cyperus rotundus* L., Bermuda grass (*Cynodon Dactylon* (L.) Pers.) *Eleusine indica* (L.) Gaertn., *Digitaria sanguinalis* (L.) Scop., and *Eriochloa polystachya* H.B.K. Sixteen plots were measured out, $20' \times 8'$, and a $4'$ section of each was hoed, spaded, and planted to *Trichachne insularis* (L.) Nees, a grass resistant to most chemical herbicides. Four stools of sugar cane were planted in each plot to determine susceptibility or resistance to

chemical treatment. Three plots were thoroughly sprayed with each of four chemical treatments, three plots were flamed with a flame thrower at intervals of three weeks, and one remained untreated.

The results were recorded at the end of 14 weeks on what seemed to us to be a fair approach to a quantitative basis. In Table 1 effectiveness of treatment is given in percentage of destruction of weeds. The percentage of plants remaining alive or arising from unaffected rootstocks or seeds was taken in relation to the number of living plants in the check plot.

TABLE 1

Weed	Percentage destruction by treatment*				
	A	B	C	D	E
Eleusine indica	100	85	98	100	100
Cynodon Dactylon	95	80	90	80	50
Digitaria sanguinalis	100	80	90	85	50
Eriochloa polystachya	90	25	80	80	0
Trichachne insularis	90	50	90	50	100
Cyperus rotundus	90	25	80	90	0

* A = 150 ml of 40% 2,4-D ester/gal of diesel oil; B = 1 lb of pentachlorophenol/15 gals of diesel oil; C = 1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, 15 gals of diesel oil; D = Dow G-502, dinitro-*o*-secondary butyl phenol, 1 gal, 1 liter of 40% 2,4-D ester, 10 gals of diesel oil; E = firing.

Effective control was obtained with 2,4-D (150 ml of 40% ester/gal of diesel oil) at the rate of 40–50 gals/acre. Two other treatments applied at the same rate per acre were very effective: (a) dinitro-*o*-secondary butyl phenol (Commercial Dow Contact) in combination with 2,4-D and diesel oil (1 gal of Dow Contact, 500 ml of 40% 2,4-D ester, and 10 gals of diesel oil) and (b) pentachlorophenol with 2,4-D and diesel oil (1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, and 15 gals of oil). Firing to destroy all above-ground parts of a grass weed stand proved to be ineffective. *Cyperus rotundus* was rather stimulated in growth. Likewise, there was stimulation in the germination of seeds of *Amaranthus spinosus* L. and other plants.

All the treatments were highly injurious to cane when the spray was applied to plants younger than 4 months of age. Older plants escaped injury when the spray was applied carefully.

More recent experiments have shown that aromatic oils (Shell) are far more effective than diesel oil, making it possible to reduce the quantity of 2,4-D ester to 75 ml/gal of oil.

It is evident that 2,4-D, which alone has little effect on grasses, becomes a very active grass herbicide when dissolved in diesel or aromatic oil and applied as a fine spray. The practical implications will be clear when one considers that grass weeds have become the most pernicious competitors of sugar cane for soil nutrients and soil moisture.

It is recognized that such an active herbicide has to be applied with extraordinary care to growing cane. If the 2,4-D oil spray is applied on a windy day, much injury may result to leaves of cane. To obviate these shortcomings of the treatment, two alternative methods of application have been developed. The first involves a preplanting application. Fields are prepared in the ordinary way, including furrowing and ditching, and irrigation water is then applied. Grass weeds are allowed to grow, and at the end of 3 weeks the 2,4-D oil spray is applied either with knapsack or power sprayers. Cane is planted the day following the herbicide application. This will considerably reduce the grass growth while the young cane is growing. Hoeing or other forms of mechanical cultivation are not eliminated altogether, but much labor saving is effected. The second method requires that the 2,4-D oil spray be applied to fields of growing cane when this is over 3 months of age. A combination of the two methods has been found to reduce effectively the number of hoeings from 6 or 7 to 3 in new plantings.

References

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Demonstration of Reducing Enzyme Systems in Neoplasms and Living Mammalian Tissues by Triphenyltetrazolium Chloride

FRANCIS H. STRAUS, NICHOLAS D. CHERONIS, and ELIZABETH STRAUS

The Presbyterian Hospital and *The Synthetical Laboratories, Chicago*

Tetrazolium salts first prepared by Pechman and Runge (*6*) in 1894 and extensively investigated by Kuhn and Jerchel (*3*) were proposed by Lakon (*4*) for testing the viability of seeds. Attention to the usefulness of these reagents was pointed out in this country by the report of Dutcher (*2*), who interrogated Lakon in 1945. Subsequently, Porter, Durrell, and Romm (*7*), Mattson, Jensen, and Dutcher (*5*), Waugh (*9*) and Cottrell (*1*), confirmed the observations of Lakon and indicated that tetrazolium salts may be useful as reagents to detect differences in the viability of seeds and other tissues.

We synthesized a series of tetrazolium salts including the 2,3,5-triphenyl and 2,3-diphenyl-5-methyl compounds. The present paper deals with the demonstration of reducing enzyme systems in neoplasms and living mammalian tissues by means of the triphenyltetrazolium salt.

Warburg and Christian (*8*), in 1943, showed that the plasma of animals bearing large sarcomata contained an increased quantity of zymohexase and postulated that, in contradistinction to normal mammalian cells, the tumor cell obtains energy from glycolysis even in the presence of available oxygen. It appeared to us that, if there were an appreciable difference in the amount of glycolysis in the neoplastic cells as compared to normal cells in contact with available oxygen, such a difference might be demonstrated by reagents capable of reacting with enzymes in the chain of glycolytic fermentation. Tetrazolium salts