Subcultures from these excised roots were made to dextrose solutions on August 20; a set of second subcultures into dextrose was made on September 23; a third, on October 22; a fourth, on November 24; a fifth, on December 26, and a sixth, on January 30, 1937. The roots which had thus grown for more than 6 months in a dextrose solution through seven transfers showed as good growth in the sixth subculture as in the original transfer to the dextrose solution. The culture solutions for the second subculture (third transfer in dextrose) were sterilized at fifteen pounds for twenty minutes, and for the third subculture part of the medium was sterilized at six pounds for thirty minutes and part at fifteen pounds for twenty minutes. No differences in growth in the media sterilized at the two different temperatures were noted.

The experiments with Cerelose were less extensive. A transfer from White's solution containing cane sugar to the same solution with Cerelose substituted for the cane sugar was made on December 26, 1936, and subcultures from these roots to the same medium on January 30, 1937. For both transfers the medium was sterilized for thirty minutes at six pounds pressure. No difference was noted in the growth in the Pfanstiehl dextrose and in the Cerelose. The same type of growth developed with both samples of dextrose.

We are puzzled to account for the differences in our results and those of White. While he does not define the conditions under which his experiments were performed it may be assumed that they were similar to those reported eariler.[2] The conditions of our experiments are similar to those given by White.

We considered the possibility that the differences in results might be because of differences in the variety of tomato used. White has used root tips from the variety Bonny Best, while our root tips were from seeds of a pink-fruited variety from Mexico, Ajo de Verrado No. 580, secured through Dr. J. W. Lesley and Dr. H. L. Blood. This tomato resembles *Lycopersicon esculentum* in some characters and *L. pimpinellifolium* in others. However, we secured root tips from seeds of the variety Bonny Best and found that they too grew in both samples of dextrose mentioned above. Though we have not subcultured these root tips through a series of transfers in dextrose solutions, there is no reason to believe that they would not continue to grow in dextrose solutions as the root tips of the other variety do. Through the courtesy of Dr. P. R. White we secured subcultures of the Bonny Best tomato roots (Clone C) which he has used. These root tips grew in a solution containing mineral salts, baker's yeast and dextrose.

Although White used heat in sterilizing his culture media we considered the possibility also of the roots growing at the expense of decomposition products of dextrose formed by heating in sterilization of the medium. It is well known that small quantities of organic acids and other products are formed in the sterilization of dextrose by heat, and there was a possibility that these decomposition products might be a factor of importance in determining the growth of tomato roots in dextrose solutions. We prepared sterile dextrose solutions by filtration through Jena fritted glass filter funnels and added the sterile dextrose to the solutions of the mineral salts and yeast which had been previously sterilized by heat. Excised root tips of the variety of tomato we have used grew in these solutions. Similar experiments with the Bonny Best variety have not yet been completed, but we have no reason to expect that the results will be different.

It appears, therefore, that excised tomato root tips are able to assimilate dextrose; and we conclude that neither the variety of tomato nor the method of sterilization of the media is responsible for the difference between our results and those of White.

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[2] P. R. White, *Plant Physiol.*, 9: 585–600, 1934.

THE ANESTHETIC EFFECTS OF SOME N-ARYL BARBITURIC ACIDS CONTAINING DYE-FORMING GROUPS

EFFECTS in physiological responses to pharmacologically active substances are often readily demonstrable, but the causes may be comparatively difficult of proof. The more objective the experiment the more reliable will be the result. The idea of introducing dye groups into physiologically active compounds in order to definitely locate the point of action, if not the mode, is apparently not a new expedient. Ehrlich and Einhorn,[1] Fulton[2] and Gardner and Joseph[3] have tested certain local anesthetics containing dye groups with varying success. Rising, Shroyer and Stieglitz[4] and Pierce and Rising[5] have attempted to do the same with barbituric acid hypnotics. The latter two tested a number of barbituric acid compounds containing dye-forming groups at the 5 carbon atom without producing anesthesia. Apparently heretofore no attempt has been made to test barbituric acid compounds with a dye-forming group attached to the nitrogen atom for anesthetic effects.

[1] P. Ehrlich and A. Einhorn, *Ber.*, 27: 1870, 1894.
[2] J. F. Fulton, *Am. Jour. Physiol.*, 57: 153, 1921.
[3] J. H. Gardner and L. Joseph, *Jour. Am. Chem. Soc.*, 57: 901, 1935.
[4] M. M. Rising, J. H. Shroyer and J. Stieglitz, *Jour. Am. Chem. Soc.*, 55: 2817, 1933.
[5] A. E. Pierce and M. M. Rising, *ibid.*, 58: 1361, 1936.

Buck[6] has recently synthesized a number of N-aryl barbituric acid derivatives, amongst which he included the following with dye-forming groups:

(1) 1-p-(phenylazo)phenyl-5,5-diethylbarbituric acid
(2) 1-p-(4-aminophenylazo)phenyl-5,5-diethylbarbituric acid
(3) 1-p-(2-azo-α-naphthol-5-sulfonic acid)phenyl-5,5-diethylbarbituric acid
(4) 1-p-(4-aminonaphthylazo)phenyl-5,5-diethylbarbituric acid
(5) 1-p-(4-hydroxynaphthylazo)phenyl-5,5-diethylbarbituric acid
(6) 1-m-(4-aminophenylazo)phenyl-5,5-diethylbarbituric acid
(7) 1-m-(2-azo-α-naphthol-5-sulfonic acid)phenyl-5,5-diethylbarbituric acid
(8) 1-m-(4-aminonaphthylazo)phenyl-5,5-diethylbarbituric acid
(9) 1-m-(4-hydroxynaphthylazo)phenyl-5,5-diethylbarbituric acid

These dyes have now been administered intraperitoneally into albino mice with the hope of inducing anesthesia.

The results obtained vary. Numbers 1, 2, 4, 6 and 8 induce true anesthesia, numbers 3 and 7 cause a mixed effect, a convulsive element apparently masking the anesthetic, and in the dosage range possible, numbers 5 and 9 are inert. Some of these compounds are amphoteric, but they are equally effective as anesthetics, whether dissolved with the aid of equivalent amounts of alkali or of acid. In general the meta isomers are more soluble than the para.

Certain animals were sacrificed during the early part of anesthesia or within an hour after injection of the various dyes. Gross anatomic examination of these animals uniformly showed, with the exception of the brains, generalized staining of the tissues, especially of the subcutaneous fat. Those dyes which induced anesthesia, however, not only stained the tissues as above but also stained the brain tissue. The other dyes stained brain tissue little if any. Apparently, therefore, there is a parallelism between the brain-staining properties of these dyes and their ability to induce anesthesia. These results promise to provide material for further investigations, such as histological studies, which are now in progress, quantitative determinations in the tissues, effects on cellular metabolism and their fates in the animal body, all of which may lead to a better understanding of the processes underlying the phenomenon of anesthesia.

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[6] J. S. Buck, *ibid.*, 58: 1284, 2059, 1936; 59: 1249, 1937.

DIFFRACTION OF X-RAYS BY BUILT-UP FILMS OF PROTEINS

THE method of building up successive layers of protein monofilms has been recently demonstrated by Langmuir, Schaeffer and Wrinch.[1] They further indicated the possibility of investigating the structure of these layers by means of x-rays. This investigation was carried out on films built up by the technique of Dr. Blodgett,[2] as modified by Clark, Sterrett and Leppla[3] in this laboratory.

A curved glass slide was used as a solid substratum on which to build the layers. Langmuir, Schaeffer and Wrinch found it preferable to employ a surface already covered with a number of layers of barium stearate, but for the purpose of the present study, in order to simplify the x-ray pattern, it was judged advisable to build up the protein layers directly on the glass itself. Layers of the AB type were added, the plate being dried between each addition of two protein layers. Only these recurrent AB films were used for the present x-ray investigation.

Preliminary x-ray photographs indicated that, unlike the built-up films of stearate, protein layers would, at the best, give only a few orders of a given spacing. They also showed a great deal of background fogging, making it advisable to adopt a vacuum camera technique.

Plates containing 30, 40 and 70 layers of egg albumin were prepared. The radiation was supplied by a Philips Metalix diffraction tube, operated at 28 KV and 20 ma., rendered monochromatic by use of a nickel foil. The sample to film distance was not obtainable in this case by the usual methods, as a curved plate was being used inside the vacuum camera designed by Schaad.[4] The photographic film was placed in two positions, a known distance apart, and two exposures made without altering the position of the sample. In this way it was possible to calculate the sample to film distance.

It was found that each sample gave a sharp diffraction arc, corresponding to a spacing of 73.3 A.U. The length of the arc indicates that this is not a highly oriented long spacing. In some cases it was possible to obtain a second order of this spacing.

According to Astbury and co-workers[5] the dena-

[1] Langmuir, Schaeffer and Wrinch, SCIENCE, 85: 76, January 15, 1937.
[2] Katharine Blodgett, *Jour. Am. Chem. Soc.*, 57: 1007, 1935.
[3] Clark, Sterrett and Leppla, *Jour. Am. Chem. Soc.*, 57: 330.
[4] J. A. Schaad, Thesis, University of Illinois, 1936.
[5] Astbury and Woods, *Phil. Trans.*, A 232, 333, 1933; Astbury and Lomax, *Jour. Chem. Soc.*, p. 846, 1936.