THE POLAROGRAPHIC CURVE OF SERUM FROM RATS FED p-DIMETHYL-AMINOAZOBENZENE[1]

WHITE and White[2, 3] have demonstrated that growth inhibition resulted in young rats when carcinogenic chemicals and certain other organic compounds were included in the diet, and they reported that this could be corrected by dietary supplements of l-cystine and dl-methionine. It was concluded that certain carcinogens, as well as non-carcinogens, could induce a specific deficiency of the sulfur containing amino acids probably by imposing on the organisms an increased requirement for organic sulfur in the form of cystine or methionine for detoxication mechanisms. Since Brdička[4] has shown that the production of the polarographic curve obtained with blood serum is quantitatively proportional to the concentration of the sulfur-containing amino acids, we used the polarograph to obtain information regarding the level of these substances in the blood of rats on diets containing p-dimethylaminoazobenzene (butter yellow). Rusch, Klatt, Dirksen and Meloche[5] have pointed out that cystine is highest in the albumin fraction of the blood and found that the height of the polarographic curve was directly proportional to the amount of serum albumin.

Twenty-four young albino rats weighing 60–70 gm were divided into two series of 12 each. Three different basic diets were used. Diet I was composed of dextrin 77, casein 6, butter 5, crisco 5, salts 4, brewers yeast 2 and cod liver oil 1. Diet II was the same except that dextrin was reduced to 67, the casein was raised to 16, and 0.02 per cent. butter yellow was added. Diet III was the same as diet I except that 0.067 per cent. cystine and 0.02 per cent. butter yellow were added. In series I, three rats were killed at the beginning of the experiment, the remainder put on diet I and kept for 21 days when 3 more were sacrificed. Butter yellow (0.02 per cent.) was added to diet 1 and after 9 days on this ration, 3 were killed and the remaining 3 placed on diet II for 7 days more after which time they were also sacrificed. Series II was performed in the same manner, except that the rats were kept on diet I for 30 days before making any changes and diet III was used instead of diet II. Blood for the polarographic determinations was obtained when the rats were decapitated and it was prepared by the method previously described.[5]

The average height of the polarographic curves of the rats on normal diets in series I was 19.7 mm, but decreased to 17.5 mm on diet I, was further lowered to 15.8 mm when butter yellow was added and increased to 17.8 after the level of casein was raised. In series II, the figures were 20 mm for the controls, 15.3 mm after 30 days on diet I, 13.3 mm after butter yellow was added and 14.8 mm when additional cystine was included in the diet. In Fig. 1 representative

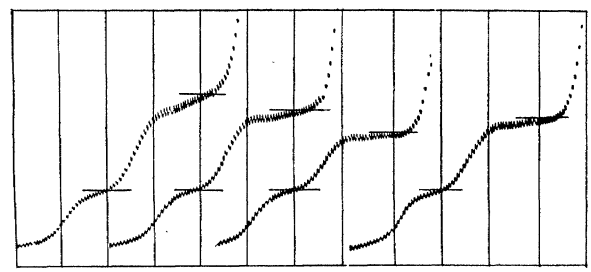

FIG. 1

curves of each group are arranged in the order just given. The lower curves found in series II were probably due to the greater depletion of cystine, since the rats were kept on the deficient diet over a longer period. The failure of the curves to return to the normal starting height may have been due to inadequate amounts of essential factors or to the short period allowed for recovery. From these results it is probable that the addition of butter yellow to the diet of rats resulted in a reduction in the level of the sulfur containing amino acids of the blood sera. This is in harmony with results obtained by different methods as reported by White and White.

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VITAMIN B_6 AND GROWTH OF EXCISED TOMATO ROOTS IN AGAR CULTURE[1]

ROBBINS and Schmidt[2] reported that pyridoxine (vitamin B_6) had a marked favorable effect on the growth of excised tomato roots in a mineral-sugar solution containing thiamin. Bonner and Devirian[3] obtained similar results. Bonner[4] reported beneficial results with five strains of tomato. Robbins[5] observed that the excised roots of one inbred tomato showed little response to pyridoxine while those of another

1 This study was supported by the Jonathan Bowman Fund for Cancer Research.
2 J. White and A. White, *Jour. Biol. Chem.*, 131: 149, 1939.
3 J. White, *Jour. National Cancer Inst.*, 1: 337, 1940.
4 R. Brdička, *Nature*, 142: 617, 1938.
5 H. P. Rusch, T. Klatt, V. W. Meloche and A. J. Dirksen, *Proc. Soc. Exper. Biol. and Med.*, 44: 362, 1940.

1 Contributions from the Department of Botany, Smith College, New Series, No. 7.
2 W. J. Robbins and M. B. Schmidt, *Am. Jour. Bot.*, 26: 149–159, 1939.
3 J. Bonner and P. S. Devirian, *Am. Jour. Bot.*, 26: 661–665, 1939.
4 J. Bonner, *Am. Jour. Bot.*, 27: 692–701, 1940.
5 W. J. Robbins, SCIENCE, 92: 416, 1940.

inbred and the F_1 heterotic hybrid exhibited considerable response. White[6] concluded that this vitamin did not improve the growth of excised tomato roots, while glycine had a marked beneficial effect.

In the experiments referred to above a liquid culture medium was used. The writer has determined the effect of pyridoxine on excised tomato roots in agar culture in experiments carried on at the New York Botanical Garden. The nutrient medium consisted of a modified Pfeffer's solution containing 1.0 per cent. sucrose and 0.5 per cent. purified agar to which thiamin, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in various combinations.

The strain of excised tomato roots was that originally isolated by Robbins and Schmidt.[7] Fragments of roots which had grown for 47 or more successive passages in a mineral-sugar solution containing thiamin or a mineral-sugar solution containing the thiazole intermediate of thiamin were used as inoculum. The inoculum was therefore in all probability free of any material other than that synthesized by the roots or contained in the solutions given. Uniform pieces of the roots growing in these liquid cultures were transferred to the agar medium in Petri dishes. These were incubated in a moist chamber at 25° C. in the dark. In subsequent passages at eight- to ten-day intervals strongly growing and uniform appearing root tips 1.0 cm in length were transferred from the medium in one Petri dish to the same medium in another. Growth was determined at the end of each passage by measuring the increase in length of the main root. Growth of branch roots was not included in these measurements. In each experiment from 12 to 31 root tips were grown on a particular medium.

In the basal medium the roots seldom grew for more than two passages. With the addition of thiamin similar root tips grew about 2.0 mm daily. In the same experiments, where pyridoxine was added to the agar medium containing thiamin, similar root tips generally showed a daily increment of 5.0–6.0 mm, or in several passages as much as 8.0 mm. Supplementing this medium with nicotinamide had no appreciable effect on the rate of growth. The further addition of neopeptone decreased the rate of growth to two thirds of that in the medium containing thiamin and pyridoxine. The addition of glutamic acid to the agar medium containing thiamin decreased the rate of growth. The addition of glycine to the agar medium containing thiamin had little or no effect.

Although the growth of the roots in the agar medium was less rapid than in the same medium without the agar, they appeared healthy and vigorous where suitable growth-substances were present. In one experiment the roots in the agar medium containing both thiamin and pyridoxine have grown for more than twenty passages during more than two hundred days with no diminution in rate of growth. The roots in the agar medium containing both thiamin and pyridoxine showed the characteristic hooks and curls noted by Robbins and Schmidt.[2]

Pyridoxine was of distinct benefit to the excised tomato roots in these experiments. Neither glutamic acid nor glycine in the amounts used appeared able to replace it.

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[6] P. R. White, *Am. Jour. Bot.*, 27: 811–821, 1940.

[7] W. J. Robbins and M. B. Schmidt, *Bot. Gaz.*, 99: 671–728, 1938.

[2] H. F. Swift, *Jour. Exp. Med.*, 33: 69, 1921.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PREPARATION OF STERILE PROTEINS IN THE "LYOPHILED" STATE[1]

IN the preparation of certain proteins (fibrinogen, thrombin) and proteinaceous material (chick embryo extract) in the lyophiled state, for uniform tissue culture media, it was noted that it was possible to obtain sterile preparations without the application of the elaborate and costly precautions necessary for aseptic technique. The sterility of these preparations was at first attributed to an empirical treatment of the dry protein with sodium-dried toluene for ten minutes at 60° C. The subsequent discovery that the method of removal of toluene after this treatment left traces of the toluene still in the material made it seem possible that it was the action of the compound over a period of some days that was effective. This was confirmed by the observation that mass cultures of bacteria in the lyophiled state were killed by the application of dry toluene for a period of four weeks, but not by treatment with toluene for ten minutes at 60° C.

The role of the lyophile process itself in the sterilizing action was not at first suspected, since this is a common method of preserving bacterial cultures.[2]

The publication of Heller's[3] study of factors involved in survival and death of bacteria in the desiccated state made it clear that lyophiling itself reduced considerably the number of organisms in a bacterial culture.

Because of the practical importance of being able to prepare, relatively easily, large quantities of sterile

[1] Aided by grants from the Rockefeller Foundation and from the Research Board of the University of California.

[3] George Heller, *Jour. Bact.*, 41: 109, 1941.