

especially those which have a relatively low virulence for experimental animals under normal conditions. Studies of this type of deficiency in experimental animals of other species are in progress.

#### SUMMARY

Riboflavin deficiency, even in a relatively early stage, greatly lowers the resistance of the rat to endemic typhus, thereby resulting in a fatal disease. Not only the serosal cells, but also the endothelial cells in several organs, notably the Kupfer cells of the liver, become greatly distended with rickettsiae under these conditions. Preliminary experiments strongly suggest that advanced vitamin A deficiency, even when making animals markedly cachectic, does not have a comparable effect. Riboflavin deficient animals remain alive for several weeks with an abnormal intracellular metabolism. This type of deficiency is worthy of further study as a possible method of approach in the investigation of other intracellular parasites and filtrable viruses. Speculation regarding the mechanism concerned also suggests the desirability of a study of the effects of this deficiency upon the production of immune bodies in general.

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#### PRELIMINARY NOTE ON THE MODE OF UNION OF THE GALACTURONIC RESIDUES IN PECTIC ACID

CONSIDERABLE progress has been made in recent years in the field of the chemistry of pectic acid, particularly through the investigations of Link and his co-workers. However, the essential points in its structure are still missing, namely, the exact position of the linkage of the individual residues of the galacturonic acid, their ring structure and the length of the chain (if pectic acid has a straight chain structure). Levene and Kreider recently limited the place of union of the individual residues to positions (4) or (5). The choice between the two has not yet been made.

For the purpose of obtaining the missing information, pectic acid has been exhaustively methylated. The product thus obtained had a composition corresponding most satisfactorily to a structure composed of approximately six units.

$C_{56}H_{90}O_{37}$	Calculated.	$OCH_3$ , 45.80
	Found.	$OCH_3$ , 45.40

On saponification the substance had the following methoxyl value:

$C_{50}H_{78}O_{37}$	Calculated.	$OCH_3$ , 34.18
	Found.	$OCH_3$ , 34.30

Whether the material is only a methylated fragment of pectic acid can as yet not be stated. The deduction as to the length of the chain of this material is tenta-

tive, inasmuch as it is based only on the methoxyl value in the fully methylated and in the saponified material. More precise information on this point is expected from the analysis of the methylated polygalactoside, obtained on reduction of the above material. This substance has now been prepared by heating the exhaustively methylated material with copper chromite catalyst in an atmosphere of hydrogen at a temperature of 175° and a pressure of 3,500 pounds per square inch during 6 hours. The product has been obtained in only a fair degree of purity, having the following composition:

$C_{50}H_{102}O_{31}$	Calculated.	$C$ 52.9, $H$ 8.1, $OCH_3$ , 48.81
	Found.	$C$ 53.8, $H$ 8.0, $OCH_3$ , 47.33

As far as can be judged from the rate of hydrolysis of the fully methylated pectic acid, the galacturonic residues seem to have a furanose structure, for after 2½ hours heating of the product with 0.01 *N* hydrochloric acid, at 100° C., about 1½ equivalents of reducing groups are developed and after 15 hours, about 3 equivalents. The methyl ester of 2,3,4-trimethyl  $\alpha$ -methyl-d-galacturonide under identical conditions remained unchanged. (R. S. Tipson.)

Thus, it seems suggestive that the galacturonic acid residues of pectic acid have a furanose structure and hence the union of the individual residues is through carbon atom (5).

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#### VITAMIN E AND NUTRITIONAL MUSCULAR DYSTROPHY

IN 1931 Goettsch and Pappenheimer<sup>1</sup> described a dietary deficiency disease in rabbits and guinea pigs characterized by dystrophy of the voluntary muscles. Morgulis and Spencer,<sup>2</sup> Morgulis, Wilder and Epstein,<sup>3</sup> found that at least two factors, both contained in whole wheat germ, were required for the prevention or cure of the disease. One factor was removed from wheat germ, previously extracted with petroleum ether, by 70 per cent. ethanol. The other factor was found in the unsaponifiable fraction of wheat germ oil. This suggested that the fat-soluble factor might be vitamin E. However, the dystrophy producing diet apparently contains a significant amount of vitamin E, since both Goettsch and Pappenheimer<sup>1</sup> and Morgulis<sup>4</sup> found

<sup>1</sup> M. Goettsch and A. M. Pappenheimer, *Jour. Exp. Med.*, 54: 145, 1931.

<sup>2</sup> S. Morgulis and H. C. Spencer, *Jour. Nutrition*, 11: 573, 1936.

<sup>3</sup> S. Morgulis, V. M. Wilder and S. H. Epstein, *Jour. Nutrition*, 16: 219, 1938.

<sup>4</sup> S. Morgulis, "Nutritional Muscular Dystrophy," Hermann and Cie., Paris, 1938.

that female rats grown and maintained on it exhibited "initial fertility" in the first and second generations. We have never observed "initial fertility" in even first generation female rats grown on purified E-deficient diets.

In this connection our attention was directed to the fat-soluble factor by the observation that the paralysis of the hind legs exhibited by adult rats on a highly purified, E-deficient diet, could be prevented by administration of a highly potent vitamin E concentrate.<sup>5</sup> Our experience confirms Goettsch and Pappenheimer's observation that young rabbits, restricted to their diet 13, develop muscular dystrophy and die. We obtained the same results when 10 per cent. of ether-extracted wheat germ was included in the diet. Morgulis<sup>4</sup> noted increased excretion of creatine by rabbits with impending dystrophy. We have recorded the weight, food consumption and creatine excretion, day by day, while rabbits were maintained on diet 13, and have established detailed criteria based on these data, which enable us to predict with considerable certainty when the attack of acute dystrophy will occur. Remedial mea-

sures were instituted a few hours before complete collapse was to be expected.

With these criteria as a basis for diagnosis we were able to produce cures in rabbits on diet 13, plus 10 per cent. of ether-extracted wheat germ, by administration of small doses of our vitamin E concentrate.<sup>6</sup> Since the results strongly suggested vitamin E as the curative factor, we next fed natural alpha tocopherol.<sup>7</sup> There was a sharp drop in urinary creatine, accompanied by increased food consumption and gain in weight. Within a few days the animals were able to move about the cages without difficulty, and the creatine output had fallen to the normal level. We have, therefore, shown conclusively that one of the factors, deficiency of which is involved in causing experimental muscular dystrophy in the rabbit, is alpha tocopherol. This study is being extended and the results will be published in detail elsewhere.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### "LUCITE" FOR MICROSCOPIC TRANSLUMINATION

"Lucite," polymerized methyl methacrylate, is a transparent resin produced by the plastics department of the du Pont de Nemours Chemical Company.<sup>1</sup> It is optically similar to fused quartz in the following ways: it has a high light transmission in the visible spectrum,<sup>2</sup> a beam of light will pass around a bend in the material with very little loss of intensity, and there is a tendency for a beam of light to become concentrated at a constriction in the material. Because of these properties it is therefore well adapted to serve as a substitute for the quartz rod in microscopic transillumination, and has the added advantages of low cost of the material and the ease with which it is machined. Leiter,<sup>3</sup> Knisely<sup>4</sup> and McClung<sup>5</sup> have given the uses and methods of construction of the quartz rod for microscopic transillumination. This is presumably the first report of the use of lucite for this purpose and as a substitute for the Abbe condenser.

#### CONSTRUCTION OF THE TRANSLUMINATING ROD

Lucite is easily turned or shaped on either a machine or hand lathe.<sup>2</sup> A piece of one half inch round stock

<sup>5</sup> C. G. Mackenzie, J. B. Mackenzie and E. V. McCollum. In press.

<sup>1</sup> The Lucite used in this work was graciously furnished by the E. I. du Pont de Nemours and Company, Plastics Department, 350 Fifth Avenue, New York City.

<sup>2</sup> Manufacturer's descriptive pamphlet on Lucite.

<sup>3</sup> S. B. Leiter, *Jour. Optical Soc. Amer.*, 11: 187-189, 1925.

<sup>4</sup> M. H. A. Knisely, *Anat. Rec.*, 64: 499-523, 1936.

twelve inches in length was used. Four inches of this length were turned to a diameter of one eighth inch on a machine lathe at moderate speed with the application of water to make the cutting smoother. A thirty degree bevel was found to be most satisfactory between the one half and one eighth inch surfaces. Following the cutting operations, the surfaces were polished with jeweler's rouge while the lathe was turning at a high speed until a smooth surface showing no turning marks was obtained. The ends of the rod were polished in the same way. It is necessary to have both ends of the rod flat-surfaced, because distorted surfaces give a field of uneven illumination which makes critical definition impossible. After the rod was completely polished it was ready for the bending operations. The small end was softened in hot oil and bent to the desired angle, care being taken not to distort the small

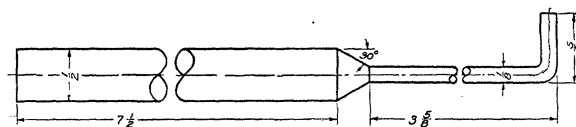


Fig. 1

light-delivering surface (see illustration). The angle of the bend can be varied to meet the special require-

<sup>5</sup> C. E. McClung, "Handbook of Microscopical Technique," Paul B. Hoeber, Inc. 2nd ed., 632-642, 1937.

<sup>6</sup> C. G. Mackenzie, J. B. Mackenzie and E. V. McCollum, *Pub. Health Reports*, 53: 1779, 1938.

<sup>7</sup> The alpha tocopherol was furnished by Merck and Co., Inc., Rahway, N. J.