have been made. While it is fully realized that caution in interpreting clinical data is essential, the present indication from the proportion of recoveries in these treated cases is that the drug has a highly beneficial effect upon the course of pneumonia. The final decision as to its real effectiveness will come only after analysis of many more treated cases than are now available.

The Institute participated in the compilation of the Eleventh Decennial Revision of the United States Pharmacopoeia, which was issued on January 1, 1936, and became official on June 1. In conformity with the policy of the U. S. Pharmacopoeial Convention, the work of revision has continued in order to keep abreast of commercial trends and therapeutic developments and to make available to all users the latest scientific information on the standardization of medicinal agents. The findings of the Revision Committee will be published from time to time as supplements to the Pharmacopoeia and will have official standing from those dates. Study of the official organic chemicals is continuing under the chairmanship of G. D. Beal with the aid of a grant from the Pharmacopoeial Convention. M. W. Green has been appointed as assistant in the Institute's department of research in pure chemistry under this grant.

Research on dental caries has been continued by the fellowship of the Buhl Foundation, which is headed by G. J. Cox with W. E. Walker and Sara F. Dixon as assistants. The study has centered upon the two fundamental aspects of nutritional control of dental caries, namely, (1) the formation of teeth immune to caries and (2) the arrest of decay in existing cavities. The nutritional factors which confer caries immunity may be, but are not necessarily, the same as those which arrest the progress of decay.

The bacteriological and serological investigations started several years ago at the suggestion of Dr. C. B. Schildecker have been continued on an enlarged scale during the year. This work, which is under the general supervision of Dr. R. R. Mellon, director of the Institute of Pathology of the Western Pennsylvania Hospital, is supported by Mellon Institute. The biochemical group (A. P. Locke, with the assistance of Rose B. Locke, Rhoda J. Bragdon and William Thompson) is concerned with the rôle of host factors in pneumococcic infections. The bacteriologists (P. B. Hadley, aided by Faith P. Hadley, F. B. Cooper, Paul Gross, Louise Peebles, L. R. Shinn and Marion L. Lewis) are investigating the development and dispensing of types I and II pneumonia serums and the mechanism of action of an antistreptococcus serum.

SPECIAL ARTICLES

PIMELIC ACID AS A GROWTH ACCESSORY FACTOR FOR A STRAIN OF THE DIPHTHERIA BACILLUS[1]

STUDIES on the nutritional requirements of certain strains of the diphtheria bacillus, which have been carried out during the last few years in this laboratory,[2,3,4,5] have served to indicate the general nature of the materials which must be supplied in order to obtain maximal growth of these organisms. In addition to suitable inorganic ions, these include (1) a readily available source of energy—*i.e.*, glycerol, ethyl alcohol, lactic acid, etc.; (2) certain amino acids, varying individually somewhat from one strain of the organism to another; and (3) one or more substances occurring in meat extract or in extractives from other tissues.

It has already been shown[4] that a boiling water extract of liver offers an adequate source of these latter growth-stimulating materials, a considerable proportion passing into the filtrate after vacuum concentration and precipitation with alcohol, and, further, that from such a solution, after removal of the alcohol in vacuo, the substances in question are readily adsorbed on charcoal and may be eluted from it with acid alcohol.[6]

This eluted material has been purified in a number of ways, keeping in mind always that more than one substance may well be involved. It was eventually found that a separation into two fractions could be accomplished, neither of which alone, in any concentration, would duplicate the effect of small amounts of the mixture with our test strain. This separation was brought about by repeatedly extracting the strongly acidified eluate with ether. The ethereal solution and the residual aqueous layer constituted the two fractions. Again, the possible multiplicity of active substances in one or both of these fractions has had to be kept in mind.

Recalling the work of Pappenheimer[7] in isolating

[1] From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston.

[2] J. H. Mueller, K. S. Klise, E. F. Porter and A. Graybiel, *Jour. Bact.*, 25: 509, 1933.

[3] J. H. Mueller, *Jour. Bact.*, 29: 515, 1935.

[4] J. H. Mueller, *Jour. Bact.*, 30: 513, 1935.

[5] J. H. Mueller and I. Kapnick, *Jour. Bact.*, 30: 525, 1935.

[6] The writer is indebted to Dr. Y. Subba Row, of the Department of Biochemistry of the Harvard University Medical School, and to the Lederle Company, Pearl River, New York, for considerable quantities of liver extract concentrates used in this work; and to Dr. Subba Row, also, for a great deal of active assistance and advice.

the "sporogenes vitamin" of Knight and Fildes,[8] the material contained in the ether extract was sought and found to be present in urine; and here again, as with the sporogenes vitamin, in considerably greater concentration in the urine of herbivors than of man. When calculated back to a liver tissue equivalent, horses' or cows' urine evidently contained a much higher concentration than liver. The isolation of the active material from cows' urine was therefore undertaken.

From one hundred gallons of cows' urine, it is now possible to report the isolation of about 0.25 gram of pimelic acid. This has been identified, first in a preliminary way by titration equivalent, carbon and hydrogen determinations and molecular weight by the camphor method of A. Rast. Identification was completed by mixed melting point determinations with commercial pimelic acid (Eastman) and by mixed melting points of the phenyl-phenacyl esters of the natural and synthetic acids. Physiological identity has been established by the completely satisfactory substitution of commercial pimelic acid for the active material of urine in growth tests with our strain of the diphtheria bacillus.

When added to a suitable control medium, pimelic acid in a concentration as low as 0.01 gamma (1×10^{-8} g) per cc of medium gives a recognizable increase in growth over the control, and a maximum effect is reached with about ten times this quantity. The control alone, containing inorganic salts, lactic acid, an acid hydrolysate of casein enriched by cystine and glutamic acid and the ether insoluble fraction of the liver eluate regularly permits rather poor, scanty growth of our test strain, amounting to about 0.8 to 0.9 mg bacterial nitrogen after three days' growth on 10 cc of the medium. This is increased by pimelic acid to more than 3.0 mg, which—grossly—is a heavy, well-developed pellicle. Increasing the concentration of pimelic acid many times, up to 1.0 mg per cc, has no further effect, either inhibitory or otherwise.

As far as the writer can learn, pimelic acid has not previously been described as a constituent of urine or of animal tissues. Naturally, the possibility exists that the active acid present in liver tissue is not identical with that isolated from urine, and if sufficient material becomes available in the course of the further study of the ether insoluble material now under way, an attempt will be made to settle this point.

A complete report of these experiments will shortly be made elsewhere.

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[7] A. W. Pappenheimer, Jr., *Biochem. Jour.*, 29: 2057, 1935.

[8] B. C. J. G. Knight and P. Fildes, *Brit. Jour. Exp. Path.*, 14: 112, 1933.

BIOLOGICAL ASSAYS FOR FLAVIN AND DERMATITIS FACTOR(S)

I. A SPECIFIC method for the assay of flavin has been found necessary to study the factors in the vitamin G complex. The following procedure was useful as a practical measure of the amount of flavin in biological materials. Albino rats (16 days old) were placed with their mother on a diet consisting of 35 per cent. casein (Labco), 56 per cent. sucrose, 5 per cent. Crisco, 4 per cent. Osborne and Mendel salt mixture and a cod liver oil concentrate (White's) supplying 20 units of vitamin A and 4 units of vitamin D per gram of diet. The rats were weaned at 21 days and placed in separate cages. An extract of rice polishings[1] (90 mg) was supplied daily to provide vitamin B_1 (6 I.U.) and the factor(s) in the vitamin G complex other than flavin. Selected dose levels of the material to be assayed for flavin were fed daily to groups of six rats. The positive control rats received 15 micrograms of pure flavin (Labco) which permitted an average growth rate of 1.5–2.0 g daily for four, six or eight weeks. Concentrated extracts of yeast have been assayed by this method.

Negative control rats showed a characteristic cessation of growth at the end of four weeks. A second type of assay was based on the recovery of these stunted animals with resumption of growth at an average rate of 2.0 g per day for two weeks when 15 micrograms of pure flavin were administered with the daily supplement. This method was more sensitive to lower levels of flavin,[2] but the time required for the complete assay was longer. Extreme depletion (5 to 6 weeks) has produced alopecia and dermal lesions which were cured in approximately four weeks with pure flavin.

II. The same two methods of assay have been employed to measure the factor(s), other than flavin in the vitamin G complex. Flavin (15 micrograms) and vitamin B_1 (6 I.U.) were furnished as daily supplements, together with selected dose levels of the test material, such as an extract of wheat. The rice polishings concentrate[3] (90 mg) as a standard control, permitted an average growth rate of 1.5 to 2.0 g daily for four to eight weeks.

In the second type of assay 30 micrograms of crystalline B_1 (Merck) and 15 micrograms of flavin were fed as daily supplements during the period (3 to 4 weeks) of depletion. The rice polishings concentrate, which also has been shown to contain the dietary

[1] C. A. Cook and R. Carroll, *Ind. and Eng. Chem.*, 28: 741, 1936.

[2] S. Ansbacher *et al.*, *Jour. Nutrition*, 11: 401, 1936.

[3] "Ryzamin-B" (Burroughs Wellcome and Co., (U. S. A.) Inc.).