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.).

extrinsic factor,[4] was then substituted for the crystalline B_1 in equivalent amounts. This concentrate (75 to 90 mg) plus the flavin produced a resumption of growth at an average rate of 2.3 to 2.6 g per day, respectively, for four weeks, while a lower dose level (56 mg) yielded 1.3 g per day for the same period. The different growth rates were not due to a variation of the vitamin B_1 intake, which was maintained at a constant level (6 I.U.).

Negative control rats supplied with only B_1 and flavin developed a progressive dermatitis in about five weeks, and several animals died. The swollen and inflamed paws have been cured by administration of the rice polishings concentrate which contained the dermatitis factor(s). The biological factor(s) was stable when the concentrate was subjected to ultraviolet irradiation but was partially destroyed by treatment in an autoclave.

With both types of assay the daily supplements were designed to provide optimal quantities of vitamins so that inadvertent additions with the test materials would not affect the growth rate beyond the biological error. Coprophagy did not become a problem. These methods are being used to assay experimental fractions in a study of the biological factor(s) which have a reputed relation to pellagra and pernicious anemia.

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THE DISTRIBUTION OF GENE FREQUENCIES IN POPULATIONS

THE effects of the various evolutionary factors can be reduced to common terms by considering the rates of change which they tend to bring about in the relative frequencies of alleles within a population. In the absence of such factors, there is a constancy of gene frequencies from the symmetry of the Mendelian mechanism. The frequency (q) of a given gene changes at the rate

$$\Delta q = -uq + v(1-q) - m(q-q_t) + \frac{q(1-q)}{2}\frac{\partial}{\partial q}\log \overline{W}$$

where u is the rate of mutation of the gene in question, v is the rate of mutation to it from its alleles, m is the effective amount of exchange between the local population under consideration and the species as a whole (gene frequency q_t), and $\overline{W}$ is the mean selective value of the array of genotypes characteristic of this population. Gene frequency is in equilibrium (stable or otherwise) at any point at which $\Delta q = 0$ except for variation due to the accidents of sampling among the gametes. The sampling variance for one generation is $\frac{q(1-q)}{2N}$ where N is the effective size of the breeding population. The pressure toward a stable equilibrium in value of q, due to mutation, crossbreeding and selection (assuming persistence of the same conditions for a long period), and the divergent tendency due to inbreeding should between them determine a certain probability distribution of values of q for the local population considered. The following formula is reached, assuming that the selective effects of the gene in question are independent of those of other genes.

$$\varphi(q) = \frac{Ce^{4N\int\frac{\Delta q\, dq}{q(1-q)}}}{q(1-q)}$$
$$= C\overline{W}^{2N} q^{4N[mq_t+v]-1}(1-q)^{4N[m(1-q_t)+u]-1}$$

More generally, selective values depend on the interactions of the entire system of genes. It is the harmonious development of all characteristics that determines the success of an organism, not the absolute grades of the separate characters and still less the composition with respect to a single series of alleles. The mean selective values, $\overline{W}$, of populations characterized by different sets of gene frequencies form a multidimensional surface which in general has many peaks. The joint distribution of the gene frequencies is given by the formula

$$\varphi(q_1, q_2 \cdots q_n) = C\overline{W}^{2N}\prod_{i=1}^{n} q_i^{4N[mq_t+v_i]-1}(1-q_i)^{4N[m(1-q_t)+u_i]-1}$$

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[4] D. K. Miller and C. P. Rhoads, *New Eng. Jour. Med.*, 211: 921, 1934.

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