THE ETIOLOGY OF PELLAGRA.

BY

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PROFESSOR WILSON's note on the etiology of pellagra,¹ published in the *British Medical Journal* early this year, tends to give a wrong impression of some recent researches in connexion with that disease. Wilson's theory of pellagra is that it follows diets in which the biological value of the proteins consumed daily falls below the value of 40 to 45 grams of meat protein, a theory developed as the result of a series of observations made in Egypt during the war.² At that time Goldberger and his colleagues of the U.S. Public Health Service had begun the long and eventually satisfactory investigation in the course of which they showed the non-communicability of pellagra, its clear association with faulty diet, and the methods, simple from a scientific point of view, required for its prevention; and their work was already lending fresh point to old theories of the dietary origin of the disease.

Wilson has shown that a number of diets on which pellagra developed contained only proteins of low biological value. The values of the different proteins are taken from Thomas's tables,³ Thomas having defined biological value as the number of parts of body nitrogen replaceable by 100 parts of the nitrogen of the foodstuff. His method consisted in remaining for a few days on a nitrogen-free diet until his nitrogen output fell to a low figure, taken to represent only endogenous metabolism, and then testing the power of various proteins, fed as additions to the nitrogen-free diet, to replace the nitrogen lost in tissue destruction.

Thomas's values, gained from experiments on himself, have not been exactly confirmed, either for man or the lower animals. Martin and Robison⁴ have pointed out how difficult it is for those using Thomas's method to avoid fallacies; for example, it is uncertain whether Thomas, previous to conducting his tests, always remained long enough on a nitrogen-free diet for his output of nitrogen to fall to a minimum and represent only endogenous metabolism. These observers attempted with great care to discover in themselves the biological value of the proteins contained in milk and whole wheat. Using Thomas's formula for their calculations, their values for whole wheat (35 per cent. C. J. M. and 31 per cent. R. R.) agree fairly well with that of Thomas (39.50 per cent.), but in the case of milk the difference was very large (51 per cent. C. J. M. and 100 per cent. Thomas). The later workers at first found that with milk nitrogenous equilibrium could not be attained until over 11 grams of milk nitrogen were taken (to replace about 2 grams of body nitrogen), thus giving a very low biological value (about 20 per cent.), but when the energy content of the diet was raised far above normal, nitrogenous equilibrium was attained at a lower level of nitrogen intake. Martin and Robison's paper gives many illustrations of the difficulties underlying determinations of this kind, and their results show the unwisdom of accepting Thomas's figures as absolute. It is remarkable that while he recorded the biological value of milk protein as 100 per cent., he recorded also large negative balances with "Frauenmilch"—cow's milk with extra sugar and cream.

Thomas's results are of interest in connexion with pellagra in that he assigned a low value to maize (29 per cent.), which fits with the earlier observation of Kossel and Kutcher,⁵ and Osborne and Harris,⁶ that zein, the principal protein of maize, is deficient in lysin and tryptophane respectively. McCollum,⁷ using Thomas's method and working with pigs, found that zein could cover 73 per cent. of the animals' nitrogen expenditure. Similar experiments were made on rats by Mitchell,⁸ who gave maize a value of 72 per cent., but found it inferior to milk and muscle.

Later, McCollum⁹ and his co-workers, taking the growth of rats as index of the biological value of a protein, assigned maize a very low position in the scale relative to

beef muscle and wheat. With the pig as the experimental animal Hart and McCollum¹⁰ found the growth value of the proteins of the wheat, maize, and oat kernels roughly equal. These growth experiments are difficult to interpret in that they are complicated by then unrecognized vitamin deficiencies.

It will be seen that it is unwise for the pellagra investigator to place implicit trust in current estimations of biological value. Actually the clinical evidence available at the moment tends to minimize the importance of protein deficiency in pellagra.

In 1920 Voegtlin, Neill, and Hunter¹¹ showed that remarkable improvement could be produced in pellagrins by the administration of a 95 per cent. alcoholic extract of ox liver in daily doses equal to 1 kilo of fresh liver. The extract was practically free from amino-nitrogen. A few years later a series of observations of a somewhat similar nature led Goldberger¹² to change his earlier view of the causation of pellagra, which coincided with Wilson's. Pellagra being a recurrent disease, it was observed that in certain asylums and institutions in the Southern United States a constant proportion of inmates tended to fall victims year by year, and it was found possible by Goldberger and his associates to study the pellagra preventive effect of alterations in the diets of certain groups.

They discovered that a liberal allowance of casein previously purified by repeated washings in acidulated water (Thomas gives casein the biological value of 70 per cent.) had no effect in preventing the recurrence of the systematic lesions of pellagra, though it had some effect in curing and preventing the characteristic dermatitis. Having at about this time accidentally noted that yeast was effective in treating and averting black-tongue in dogs (I can find no reference in Goldberger's work to the statement, put forward by Wilson, that yeast is operative only in the early stages of black-tongue, while lean meat will cure advanced cases), Goldberger began successfully to use it as a pellagra preventive and in the treatment of developed cases. The yeast used by him was a proprietary preparation—"Yeast Vitamin Harris Powder," which is a dried acidulated watery extract prepared "according to a somewhat modified Osborne-Wakeman technique," and containing very little protein nitrogen.¹³

The success of this yeast preparation (in doses of 15 grams per day) in preventing and treating pellagra led Goldberger to postulate a non-protein P.P. (pellagra preventive) dietary factor in yeast and in other foodstuffs possessing similar preventive properties. These foodstuffs were found to be mostly those containing animal protein, tomato juice being an exception.

Recently Professor Tschérkès, of the Department of Nutritional Research in Russia, has obtained results in the treatment of pellagra, not yet fully recorded, which confirm some of Goldberger's observations. In 1929 a severe outbreak of pellagra occurred in the Ukraine, the drought of 1927 and 1928 having destroyed all crops except maize. A daily allowance of 1.5 litres of milk, 200 grams of meat, or 45 grams of yeast, was found to clear up symptoms in five to six weeks.

Black-tongue in dogs, which owes its name to superficial areas of necrosis on the tongue, appears to have a geographical distribution in the United States similar to that of pellagra, which it resembles in involving a disturbance of the alimentary tract—stomatitis and diarrhoea. It can be produced experimentally in dogs by diets similar to those associated with pellagra in man and cured or prevented by foods containing abundance of the P.P. factor. Doubt has been thrown on the parallelism of experimental black-tongue and pellagra by the production in dogs by Underhill and Mendel¹⁴ of an apparently identical condition which does not respond to treatment with yeast. Underhill and Mendel's syndrome is curable by butter (found useless against pellagra and Goldberger's black-tongue) and by crystallized carotene, which has since been shown to be closely allied to, or a possible precursor of, vitamin A.¹⁵ Since the basal diets used in their experiments were lacking in this vitamin, it is possible that Underhill and Mendel's syndrome had its origin in vitamin A deficiency.

In 1926 Goldberger and Lillie¹⁶ observed failure of growth and a pellagra-like condition in the albino rat when highly purified diets devoid of the P.P. factor were fed. This pellagra-like condition, whose most striking feature is a symmetrical dermatitis, was subsequently produced in other laboratories (Chick and Roscoe,¹⁷ etc.). The external appearances of this condition resemble human pellagra, though the appearance of the lesions does not seem to be accelerated by exposure to strong light. Diarrhoea is not usual in the rat. Findlay¹⁸ has recorded stomatitis. The factor in whose absence rats fail to grow and often develop symmetrical dermatitis is called in this country vitamin B₂ (in the United States vitamin G). It has generally been supposed to be identical with Goldberger's P.P. (pellagra preventive) factor.

Later Aykroyd and Roscoe¹⁹ showed that the distribution of vitamin B₂ (rat factor) was roughly similar to that of Goldberger's P.P. factor and to the black-tongue preventive factor.

Comparison of the Effects of Various Foodstuffs on Vitamin B₂ Deficiency in Rats, on Pellagra in Man, and on Black-tongue in Dogs.

Material.	Rat Vitamin B ₂ .	Man P.P. factor.	Dog Black-tongue preventive factor.
Maize endosperm	Very poor	Associated with pellagra in U.S.A. and Italy	—
Wheat flour	Very poor	Associated with pellagra in U.S.A.	Used in basal diet to produce the disease.
Whole maize	Poor; 50% less than normal growth	Traditionally associated with pellagra	No preventive action, used in basal diet to produce the disease.
Whole wheat	Poor, but better than maize	—	66% in diet delayed onset of the disease; no protection.
Peas	30-47% needed for good growth ("Clipper" peas)	30-35% (168 gram daily); incomplete protection (Virginian cowpea)	58%; slight protection, 73%; almost complete protection (Virginian cowpea)
Wheat germ	15-30% good growth	30%; protection almost complete	31%; protection almost complete.
Fresh milk	5-10% milk solids, good growth	20% solids (1,230 g. buttermilk daily) protected	250-300 c.c. skim milk daily protected.
Lean meat	7-10% (dry wt.) good growth	12% (dry wt. 50 g. daily) protected	11% (dry weight) protected.
Dried yeast	1-3% good growth	7% (30 g. daily) protected	5% protected.
Liver	1-2% (dry wt.) ox liver, good growth	Alcoholic extract of ox liver effective in treatment	11% dried pork liver protected.
Dried egg yolk	7-15% good growth	—	17% protected.

The results for pellagra and black-tongue are taken from the work of Goldberger, Voegtlin, and others. Estimations of both the P.P. factor and the black-tongue preventive factor are approximate, since in few cases was any attempt made to discover the minimum necessary for protection. The amounts given in all cases are expressed as the percentage of the dry weight of the diet. Goldberger's clinical experiments on pellagra prevention were carried out in institutions under very carefully regulated conditions. Apart from the test foods the diet offered to the potential pellagrins was kept very constant throughout the investigation.

In the series of experiments from which the results in the table are drawn, over 60 per cent. of control rats on a basal diet, free from vitamin B₂ but otherwise complete, developed pellagra-like symptoms. The rat, however, does not develop these symptoms on diets similar to those which are associated with pellagra in man and experimental black-tongue in dogs, and is, indeed, able to perform some growth and remain free from obvious symptoms on such diets. Only a very highly purified food mixture will induce rat "pellagra," and at present the appearance of symptoms is somewhat intermittent. Experiments on rats cannot therefore prove pellagra to be a deficiency disease, but the fact that the rat's needs for vitamin B₂, set, perhaps, on a low scale, are satisfied by various foodstuffs in their order of potency as regards the P.P.

(pellagra preventive) factor and the black-tongue preventive factor, is suggestive of the identity of the three factors.

The relatively high vitamin B₂ value of one sample of whole maize employed in our experiments (another sample of maize gave a lower value) is used by Wilson to calculate the vitamin B₂ value of various human diets. Calculation on this basis gives all diets containing much whole maize a fairly high vitamin B₂ value. It is not legitimate, however, to argue, as Wilson does, that because a diet containing 65 per cent. of maize endosperm gives subnormal growth, a diet containing 100 per cent. will produce normal growth. Maize endosperm should be reckoned a negligible source of vitamin B₂.

It is dangerous to put confidence in calculations either of the biological value of the proteins of a human dietary from Thomas's figures, or of its vitamin B₂ value from such observations on rats as now exist. Thomas's values need confirmation, and the distribution of the rat factor is not completely known. Human dietaries are rarely described with exactitude—for example, it may make a considerable difference whether the term "meat" includes offal, or whether it represents sheep, ox, or horse muscle. So far vitamin B₂ has been found most abundantly in foods containing animal protein—that is, eggs, milk, meat, liver, etc.—of high "biological value." Human dietaries are therefore liable to coincide roughly as regards their vitamin B₂ value and the biological value of their proteins. Many diets associated with pellagra, as Wilson says, are low in both respects.

The vitamin (as against the protein) deficiency theory of pellagra rests on the proved possibility of preventing and curing the disease with non-protein substances. The failure of casein to give complete protection against the disease in man and the production in the rat of pellagra-like symptoms on a diet containing this "good" protein, point in the same direction.

It remains to be seen whether the distribution of vitamin B₂ in foodstuffs when known more completely will explain the epidemiology of pellagra in man. Maize was found poor in vitamin B₂ (relative to other foodstuffs), and most pellagra is associated with a high consumption of maize. The equally low vitamin B₂ content of white wheat flour fits in with various observations in the United States of the occurrence of pellagra in districts where wheat was the principal cereal in use. On the other hand, it raises the question why pellagra does not occur elsewhere in ill-nourished populations on a wheat flour staple—for example, in Newfoundland or Labrador, or the East End of London. Complete acceptance of the vitamin hypothesis involves an explanation, in terms of that hypothesis, of the non-occurrence of pellagra in millet-eating countries and its very rare occurrence on rice. Experiments are being carried on to elucidate these points.

While the whole question is still open, the evidence at the moment seems decidedly against the theory of amino-acid deficiency. Investigation of the distribution and properties of vitamin B₂ seems a promising line of attack, though at present it would be premature to regard vitamin B₂ deficiency and pellagra as synonymous.

REFERENCES.

- Wilson: *British Medical Journal*, 1930, i, 101.
- Wilson, W. H., and Roaf, H. E. (1918): Report of a Committee of Inquiry regarding the Prevalence of Pellagra among Turkish Prisoners of War (Egypt), App. VIII, 56-60, etc.
- Thomas: *Arch. Physiol.*, 1909, 219.
- Martin and Robison: *Biochem. Journ.*, 1922, 16, 407.
- Kossel and Kitcher: *Zeit. f. Physiol. Chem.*, 1900, 31, 165.
- Osborne and Harris: *Journ. Amer. Chem. Soc.*, 1905, 25, 853.
- McCollum: *Amer. Journ. Physiol.*, 1911, 29, 210.
- Mitchell: *Journ. Biol. Chem.*, 1924, 58, 905.
- McCollum, Simmonds, and Parsons: *Ibid.*, 1921, 47, 235.
- Hart and McCollum: *Ibid.*, 1914, 19, 373.
- Voegtlin, Neill, and Hunter: U.S. Public Health Reports, *Hyg. Lab. Bull.*, No. 116.
- Goldberger and Tanner: *Ibid.*, 1925, 40, 54.
- Goldberger, Wheeler, Lillie, and Rogers: *Ibid.*, 1926, 41, 257.
- Underhill and Mendel: *Amer. Journ. Physiol.*, 1928, 83, 589.
- Collison, Hume, Swedley-MacLean, and Smith: *Biochem. Journ.*, 1929, 23, 4, 634.
- Moore: *Ibid.*, 1929, 23, 4, 803.
- Goldberger and Lillie: U. S. Public Health Reports, 1926, 41, 1925.
- Chick and Roscoe: *Biochem. Journ.*, 1927, 21, 698.
- Findlay: *Journ. of Bact.*, 1928, 31, 353.
- Aykroyd and Roscoe: *Biochem. Journ.*, 1929, 23, 483.