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Nitrogen Balance in Experimental Human Deficiencies of Methionine and Cystine.*

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The studies reported herewith form part of a program which was designed to throw light on the amino acid requirements of man. We have previously reported[1,2] that human subjects deficient in tryptophane or in lysine were unable to maintain their nitrogen balance, indicating that these amino acids were essential in human diets. Observations on male subjects on a diet deficient in arginine[3] indicated that, for the male at least, arginine is a dietary essential, normal spermatogenesis being maintained only when arginine was present in the diet.

The present report deals with observations on nitrogen balance in subjects deficient in one of the sulfur-containing amino acids—methionine and cystine. Three experiments were carried out on each deficiency, 2 of them on males and one on a female subject. Each subject was kept upon the deficient diet for a period of 24 days. This was preceded by a control period of 4 days on an identical diet supplemented with the missing amino acid. A similar control period of 8 days followed the deficiency period. As in our previous studies the experimental diet consisted of fats, sugar, and certain fruits and vegetables selected for their low nitrogen content. These fruits and vegetables supplied approximately 10% of the daily nitrogen requirement, the remainder being supplied by an amino acid mixture. The daily amount of this mixture was adjusted to the weight of the subject such that his total nitrogen intake was maintained at 0.1 g per kilo per day, both in the deficient and the control periods. The remaining portion of the diet, consisting of natural foods poor in protein was not adjusted to the weight of the subject, but for the sake of convenience in preparing the diet an identical quantity was given to each subject. This resulted in a relatively small variation in total calories with the weight of the individual (total calories varied from 2400 to 2700 per day); consequently, the lighter subjects received more calories per kilogram, a fact which must be borne in mind in interpreting the results.

The amino acid mixture deficient in methionine was prepared from an acid hydrolysate of casein by a modification of the Toennies oxidative procedure,[4] which converts methionine into the biologically inactive sulfone; the latter was removed by precipitation as the insoluble calcium salt. The resulting amino acid mixture, free from methionine, was fortified by the addition of 1.5% tryptophane to replace that destroyed in the hydrolysis; 1% cystine was also added because of the low concentration of the latter amino acid in casein. The final mixture was tested on rats and was found to be growth-promoting when supplemented with methionine.

The cystine-deficient diet was prepared by passing a neutralized acid hydrolysate of casein through a filter precoated with norit A. This has been found to selectively adsorb cystine,[5] the resulting filtrate of our

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1 Holt, Albanese, Brumback, Kajdi and Wangerin, PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 726.

2 Albanese, Holt, Brumback, Hayes, Kajdi and Wangerin, PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 728.

3 Holt, Albanese, Shettles, Kajdi and Wangerin, *Fed. Proc.*, 1942, **1**, 116.

4 Toennies, G., *J. Biol. Chem.*, 1942, **145**, 667.

5 Weidinger, A., *Rec. Trav. Chim. Pays Bas*, 1937, **56**, 562. Also unreported experiments of this laboratory.

product containing only 0.12% of this amino acid. The cystine-deficient mixture was fortified with 1.5% tryptophane to replace that destroyed during hydrolysis.

The control diets used in the pre- and post-deficiency periods were prepared as above described, the methionine poor diet being supplemented by 3% dl-methionine, and the cystine-poor diet by 1% l-cystine to provide a complete amino acid mixture.

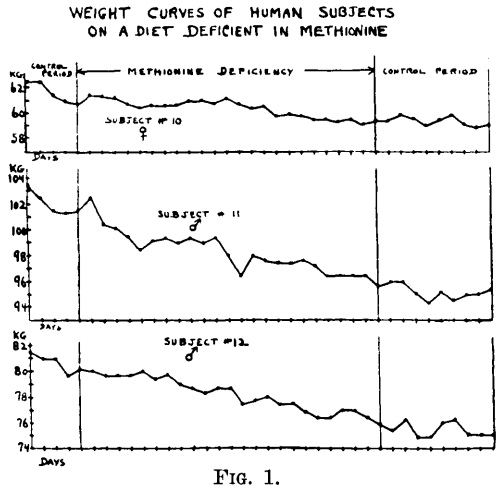

FIG. 1.

Results. The 3 subjects on the methionine-poor diet exhibited no symptoms or signs of disease during the deficiency period. They lost a small amount of weight as is shown by their weight curves (Fig. 1). Various laboratory studies were made upon them which will be reported at a later date. Only the data on nitrogen balance are presented here (Fig. 2). It will be noted that both male subjects developed negative nitrogen balances promptly after the methionine poor diet was started, and that they remained in negative nitrogen balance until the diet was supplemented with methionine. The female subject showed a minimal negative nitrogen balance during the deficiency period followed by a positive N balance when the diet containing a methionine supplement was

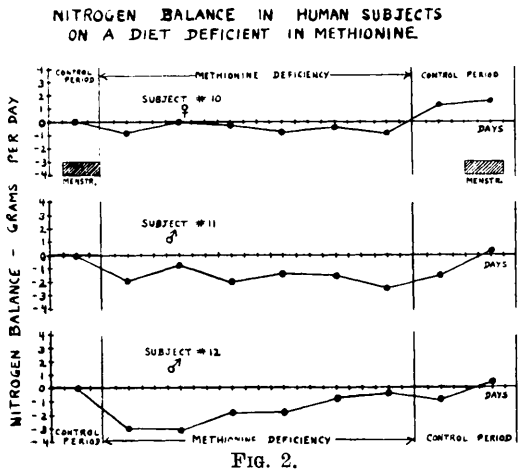

FIG. 2.

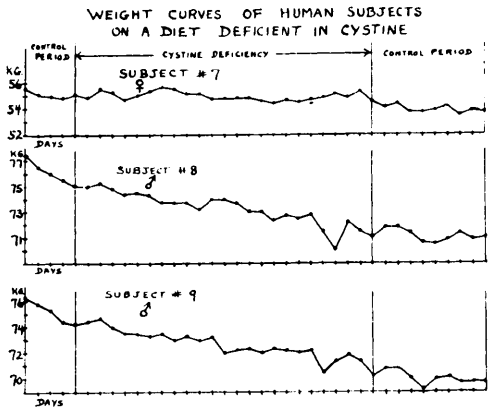

FIG. 3.

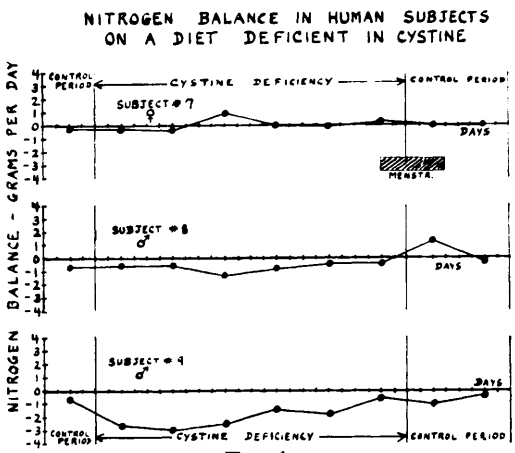

FIG. 4.

given. In interpreting her balances we must point out that she weighed less than the male subjects and consequently received more food per kilogram of body weight. Although her nitrogen intake in terms of body weight was practically identical with that of the 2 males she received relatively more carbohydrate and fat which would have brought about some degree of protein sparing. The data on these 3 experiments indicate that methionine

must be present in the diet in order to maintain nitrogen equilibrium and that this amino acid may be regarded as a human dietary essential.

Weight curves and nitrogen balances on 3 subjects on a cystine-deficient diet are given in Figs. 3 and 4. It will be noted that the female subject weighing 55 kg maintained her weight and remained in nitrogen equilibrium throughout the experiment. The 2 male subjects, both of whom weighed 75 kg at the outset, lost weight throughout the deficiency period. One of them showed a definite negative nitrogen balance during the deficiency period with a return to nitrogen balance in the post period when the cystine containing diet was substituted. The other subject showed only a minimal negative N balance during the deficiency with some indication of a rise in N retention in the post-period. We do not feel justified in concluding from these experiments that cystine is a human dietary essential. Although the evidence is somewhat suggestive that this may be the case, it would seem desirable to obtain confirmatory data from further experiments in which the energy quotient is constant for all subjects and protein sparing effects are eliminated.

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Enumeration and Differentiation of Leukocytes in the Counting Chamber with Propylene Glycol-Aqueous Stains.*

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The hemolytic and solvent properties of propylene glycol suggest its use as a white blood cell diluting fluid and staining base.

Noting the occurrence of hematuria in animals following the administration of various glycols,[1,2] Von Oettingen and Jirouch[3] demonstrated that the glycols exert a hemolytic action *in vitro*. Lehman and Newman[4] in 1937 mixed propylene glycol with water, thereby discovering that all concentrations produced immediate hemolysis of rabbit blood. Similar observations with human blood were made independently by Weatherby and Haig.[5] The effect of these mixtures on the white blood cells was not mentioned.

The following observations were made by the author. Freshly drawn human blood was mixed one part in 20 parts with equal proportions of propylene glycol and water and with a concentration of 55% propylene glycol and 45% distilled water. Within 5 min the red blood cells became almost invisible, a few remained in the background but in no way interfered with the counting of the white blood cells. Leukocyte counts remained within the limits of normal variation for a period of 60 hr. (Table I.)

The mixture of propylene glycol and water in these proportions possessed many desirable features as a white blood cell diluting fluid. Its increased viscosity compared with aqueous solutions was a distinct advantage; in filling the pipette the end point was reached more accurately, there was less settling and clumping after standing in the pipette and there was less tendency to over-

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1 Bachem, C., *Med. Klinik.*, 1917, **13**, 7.

2 Page, I. H., Coryllos, P., *J. Pharm. and Exp. Therap.*, 1926, **27**, 189.

3 Von Oettingen, W. F., and Jirouch, E. A., *J. Pharm. and Exp. Therap.*, 1931, **42**, 355.

4 Lehman, A. J., and Newman, H. W., *J. Pharm. and Exp. Therap.*, 1937, **60**, 312.

5 Weatherby, J. H., and Haig, H. B., *J. Am. Pharmaceut. Assn.*, 1938, **27**, 466.