of life, and another to perform a similar function as it affects more direct clinical observation and diagnosis of human illness.

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SPECIAL ARTICLES

THE VITAMIN CONTENT OF CASEIN[1]

As the techniques of nutritional investigations become more refined, it is increasingly important to know the vitamin content of the experimental diets with considerable accuracy. The fat, inorganic salt mixture and sucrose present in a typical purified diet carry at most only traces of vitamins as determined by micro-analytical methods. However, the casein, which is the usual protein source, must be carefully purified, and even after purification it may carry detectable amounts of vitamins. In order to obtain a quantitative measure of the amount which casein may carry, assays for seven of the B-vitamins were made on several representative samples of casein.

The casein samples were: (1) crude commercial casein,[2] (2) alcohol extracted casein, (3) acid washed casein, (4) Labco casein,[2] and (5) Smaco casein.[3] The alcohol extracted and acid-washed casein are routinely prepared in this laboratory from crude commercial casein according to the following methods.

Acid-washed casein: Four and a half kg of crude casein are placed in a 20-gallon crock and 65 liters of water are added slowly with continual stirring to avoid formation of lumps. Fifty ml of 1 N HCl is stirred into the mixture and allowed to stand at least 30 minutes. The aqueous layer is syphoned off and the crock refilled with fresh water. Again 50 ml of 1 N HCl is stirred in and the casein allowed to settle for 30 minutes. This procedure is repeated for a total of 8 extractions. The crock is refilled with 65 liters of fresh water and 1 liter of 1.2 N NH_4OH is stirred in. The mixture has the consistency of a thin paste at this point. One N HCl is added slowly with continual stirring from top to bottom until the casein coagulates (pH 4.6). When the casein has settled out, the liquid is syphoned off and the precipitated casein is placed in a cheese-cloth bag. Hot water is run through the mass until it reaches a temperature of 50–60° C. The water is expressed from the casein with a hand press. After final drying on trays at a temperature not exceeding 60° C. the casein is ground in a suitable mill.

TABLE I

THE VITAMIN CONTENT OF CASEIN SAMPLES
(The values are expressed as mgm per 100 gm of casein)

	Thiamine	Riboflavin	Niacin	Pyridoxine	Panto-thenic acid	Biotin*	Folic acid	
							S. faecalis	*L. casei*
Crude	0.120	0.360	0.340	0.070	0.450	6.00	0.019	0.013
Alcohol extracted	0.013	0.160	0.085	0.027	0.080	1.79	0.006	0.006
Acid washed	0.023	0.026	0.038	0.012	0.057	1.35	0.003	0.003
Labco	....	0.120	0.074	0.008	0.037	...	0.004	0.008
Smaco	0.016	0.100	0.036	0.026	0.053	0.93	0.008	0.009

* Expressed as micrograms per 100 grams of casein.

Alcohol extracted casein: A glass-lined pressure cooker with a built-in filter and mechanical stirrer is used. The cooker is charged with 60 liters of 95 per cent. ethanol to which is added with stirring 20 kg of crude casein. The stirring is continued for 2 hours while the temperature is maintained at 75 to 85° C. under pressure. The alcohol is filtered off while hot and a fresh charge of 95 per cent. ethanol is added. The extraction is repeated three times. The casein is thoroughly dried at about 50° C.

Thiamine was determined by the thiochrome method of Hennessy,[4] riboflavin by the fluorometric method of Connor and Straub[5] as modified by Andrews,[6] niacin by the microbiological method of Snell and Wright[7] as modified by Krehl, Strong and Elvehjem,[8] pyridoxine by the yeast growth method of Atkin *et al.*,[9] pantothenic acid by the method of Neal and

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Obtained from the Borden Company, 350 Madison Avenue, New York 17, New York.

[3] Obtained from the SMA Corporation, Chagrin Falls, Ohio. We are indebted to the following individuals who cooperated in this study: G. W. Newell, B. S. Schweigert, W. A. Krehl, L. Glasinovic, Margaret Ives, Anne E. Pollard and Lillian Alberty.

[4] D. J. Hennessy, *Cereal Chemists' Bulletin*, 2: 1, 1942.

[5] R. T. Connor and G. J. Straub, *Ind. Eng. Chem., Anal. Ed.*, 13: 385, 1941.

[6] R. S. Andrews, *Cereal Chem.*, 20: 3, 1943.

[7] E. E. Snell and L. D. Wright, *Jour. Biol. Chem.*, 139: 675, 1941.

[8] W. A. Krehl, F. M. Strong and C. A. Elvehjem, *Ind. Eng. Chem., Anal. Ed.*, 15: 471, 1943.

Strong,[10] biotin by the method of Shull, Hutchings and Peterson,[11] as modified by Shull and Peterson,[12] and folic acid as measured by both *L. casei*[13] and *S. faecalis*.[14] The results are given in Table 1.

It is evident that the four purified caseins carry significantly smaller quantities of all the vitamins studied than the crude casein. The actual amount varies from 1/10 to 1/2 of that present in the unpurified casein. The vitamin content of the four purified caseins is approximately the same except in the case of acid-washed casein, which showed a lower content of riboflavin. The purified caseins used in this study therefore can not be considered vitamin-free. The amount present may not affect the experimental results in most cases, but the amount supplied must be taken into account when vitamin requirements are calculated. The data presented in Table 1 are merely results for specific samples of casein and can not be used for routine calculations since each batch of casein may vary considerably.

While only certain known vitamins have been determined, it is probable that other unknown compounds stimulating the growth of experimental animals may be carried by purified casein. Although the vitamin content of alcohol extracted casein tended to be somewhat higher than that of the acid washed, studies with guinea pigs,[15] dogs and rats receiving sulfa drugs have indicated that alcohol-extracted casein may contain less of certain unknown nutritional factors.

The problem of obtaining a suitable protein source completely free of all growth-stimulating substances other than the essential amino acids has not been solved. The substitution of synthetic amino acids for the natural protein in experimental diets may be one means of solving this problem, but this can not be done without studying the effect of this change on the requirement of other factors.

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[9] L. Atkins, W. L. Williams, A. S. Shultz and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, 16: 67, 1944.

[10] A. L. Neal and F. M. Strong, *Ind. Eng. Chem., Anal. Ed.*, 15: 654, 1943.

[11] G. M. Shull, B. L. Hutchings and W. H. Peterson, *Jour. Biol. Chem.*, 142: 913, 1942.

[12] G. M. Shull and W. H. Peterson, *Jour. Biol. Chem.*, 151: 201, 1943.

[13] T. D. Luckey, G. M. Briggs, Jr., and C. A. Elvehjem, *Jour. Biol. Chem.*, 152: 157, 1944.

[14] T. D. Luckey, G. M. Briggs, P. R. Moore, C. A. Elvehjem and E. B. Hart. (In press.)

[15] M. D. Cannon, G. J. Mannering, Marie Zepplin, C. A. Elvehjem and E. B. Hart, *Arch. Biochem.*, 7: 55, 1945.

THE RELATION OF ENDOCRINE GLANDS TO THE GASTRIC SECRETORY DEPRESSANT IN URINE (UROGASTRONE)[1, 2, 3]

THE purpose of this preliminary communication is to present results of experiments on the origin of urogastrone, a gastric secretory depressant in urine, and its relationship to certain of the endocrine glands.

The work to be reported here is part of a systematic investigation of the effect of certain endocrine glands on the production of urogastrone. Three of these endocrine glands, namely, thyroids, ovaries and pituitaries, have been removed to date from different series of dogs and collections of urine made from these animals.

Urine was collected from each of the following series of female dogs: six normal, six oöphorectomized, six thyroidectomized plus oöphorectomized, and separately from two dogs that were hypophysectomized. The transbuccal method for removing the hypophysis was employed on the latter two. Autopsies of these two dogs revealed that the hypophyses were removed. Serial sections of the hypothalamic regions of each brain were also examined and found to be normal.[4]

Urogastrone was prepared by the procedure used in Ivy's laboratory,[5] a modification of the Katzman-Doisy method originally employed by Sandweiss, Saltzstein and Farbman[6] in obtaining their urine extracts of pregnant and of normal women.

The five preparations of urogastrone made from these four series of animals were each tested for their effect on gastric secretion stimulated by histamine in both Heidenhain pouch and gastric fistula dogs. The dogs were fasted at least 24 hours before each experiment.

Between 25 and 53 gastric secretory (double hista-

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[3] The valuable surgical assistance of Dr. J. Thorstad and others of Harper Hospital during the early phases of this study is gratefully acknowledged.

[4] The authors wish to acknowledge with appreciation the aid given by Dr. Gabriel Steiner, professor of neuropathology at Wayne University College of Medicine, for his examination of the hypothalamic regions of the brain and the decalcified bases of the skull.

[5] J. S. Gray, E. Wieczorowski, J. A. Wells and S. C. Harris, *Endocrinology*, 30: 129, 1942.

[6] D. J. Sandweiss, H. C. Saltzstein and A. A. Farbman, *Am. Jour. Dig. Dis.*, 5: 24, 1938.