at the aeronautical laboratory, which has now been given the status of a division, and to investigations in industrial chemistry, including that on unshrinkable wool, on wool wax, utilization of minerals and on producer gas investigations, particularly the suitability of Australian hardwoods for the production of charcoal. Substantial progress has been made in building up a research organization and the development of research methods for dealing with lubrication, bearing and wear problems in Australia. The work is undertaken as cooperative research with the University of Melbourne and housed in the new chemistry school. The work of the Dairy Research Section has included a survey of the properties of Australian butter and the storage and transport of butter fat without refrigeration.

SPECIAL ARTICLES

SUBCLINICAL VITAMIN DEFICIENCY[1]

I. TISSUE ANALYSES

INDIVIDUALS subsisting upon inadequate diets generally experience a more or less prolonged period of ill-health before unequivocal symptoms of deficiency make their appearance. Recognition of their deficiency in this subclinical stage is difficult because of the vagueness and generality of their complaints. Speculation on the extent to which the general population may be affected has led therefore to a program designed to decrease the incidence of latent deficiency by increasing considerably the consumption of vitamins.

Definition of "normal vitamin requirements" has proved, however, to be a complicated problem. The suggestion that our civilized diet is not normal has made impossible the customary identification of "usual" with "normal." The essentially intracellular character of the enzymes derived from vitamins,[2] and the poorly understood equilibria between these enzymes and the body fluids, have made difficult the interpretation of vitamin analyses of blood, urine and stools. Before these indirect measurements of nutritional status can be used critically, a primary knowledge of the tissue concentrations which they reflect must be obtained.

The type of information required can be illustrated by Fig. 1. A classical curve of enzyme action is used to represent the probable relationship between tissue thiamin concentrations and the tissue functions dependent upon thiamin enzymes. The problem is to identify the position of points M, m and S, and to determine the thiamin intake necessary to maintain the concentration of enzyme at or near M under varying conditions of energy output, environmental temperature and metabolic mixture. Solution is complicated by the existence of different relationships in different tissues and in different age groups, Tables I and II.

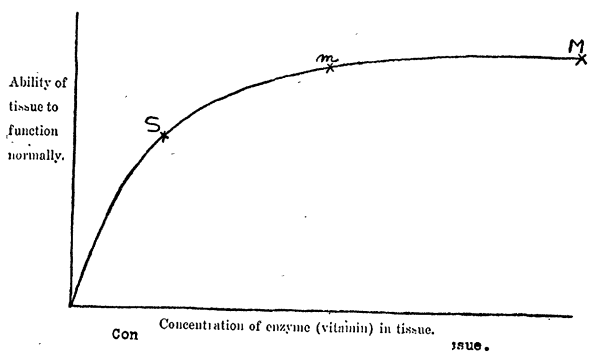

FIG. 1. Scheme of the relationship between tissue thiamin concentrations and the ability of tissues to carry on their normal functions. M = Maximum concentration of enzyme obtainable in tissues. m = Minimum concentration of enzyme compatible with normal function. S = Concentration of enzyme at which symptoms of deficiency appear.

TABLE I

SKELETAL MUSCLE THIAMIN IN DIFFERENT AGE GROUPS OF PRESUMABLY SIMILAR NUTRITIONAL STATUS

Age	Micrograms of thiamin per gram of muscle
6 month fetus	1.4
8 month fetus	1.5
Term infant	1.3
1 year	1.6
5 years	1.0
10 years	1.2
30 years	0.4
37 years	0.4
48 years	0.5
51 years	0.4

Tissues from both post-mortem and biopsy tables are available for vitamin assays. Post-mortem material permits analysis of the relationships between changes in vitamin concentrations in different tissues. Its assay is therefore a necessary preliminary to the evaluation of observations made on single tissues. Biopsy specimens are usually restricted to one tissue but are free from the unspecific effects of terminal illness and have the advantage of permitting repeated observations on ambulatory subjects. From studies of biopsy and post-mortem material a fair composite picture of the relationship between changes in vitamin

[1] Aided by grants from the R. R. Williams and R. E. Waterman Fund for the Investigation of Nutritional Disease, Research Corporation, New York City. Part II—"Micro Muscle Biopsies" and Part III—"The Thiamin Content of Micro Muscle Biopsies" were aided by a grant from the National Research Council, Washington, D. C.

[2] E. A. Evans, Jr., "The Biological Action of the Vitamins." The University of Chicago Press, Chicago, 1942.

TABLE II

CEREBRAL CORTEX THIAMIN IN DIFFERENT AGE GROUPS OF PRESUMABLY SIMILAR NUTRITIONAL STATUS

Age	Micrograms of thiamin per gram of cortical tissue
8 month fetus	0.5
Term infant	0.3
6 weeks	0.6
7 weeks	0.6
6 years	1.3
6 years	1.0
30 years	1.1
51 years	1.0
58 years	1.1

intake and changes in tissue vitamin concentrations may be constructed.

Preliminary work of this kind[3,4] has indicated that the thiamin content of skeletal muscle may serve in man as an index of thiamin nutrition. Changes in muscle thiamin in general parallel changes in other tissues, Table III. Since muscle is readily available

TABLE III

CONCENTRATION OF THIAMIN IN HUMAN TISSUE (MICROGRAMS PER GRAM) PARALLELISM BETWEEN CONCENTRATION IN SKELETAL MUSCLE AND CONCENTRATIONS IN OTHER TISSUES

Patient	Nutritional status	Heart	Liver	Kidney	Brain	Skeletal muscle
A	Good	2.3	1.1	1.7	1.0	0.4
B	Fair	1.3	1.0	1.2	..	0.2
C	Poor	0.6	0.3	0.4	0.5	0.0
D	Alcoholic Polyneuritis	Generalized symptoms: heart failure, jaundice, neuritis				0.2
E	Alcoholic Polyneuritis	Generalized symptoms				0.2
E	2 days after cessation of vitamin therapy	No symptoms				0.6

for biopsy, techniques can be developed which make repeated muscle analyses clinically feasible.[5,6] These techniques should facilitate solution of the general problem posed in Fig. 1 and furnish information concerning the frequency and severity of subclinical thiamin deficiency among the general population. Their extension to other vitamins is being investigated.

II. MICRO MUSCLE BIOPSIES

Investigation of a number of clinical problems—nutritional, metabolic and dystrophic—can be facilitated by a ready supply of skeletal muscle. The needle arrangements described by Silverman[7] can be used for muscle biopsies. For this purpose, the bevel of both the inner and the outer needle should be increased somewhat and the edges of the outer brought to a razor sharpness by honing on a hand stone.

Samples of muscle by this technique run between 5 and 15 mg, wet weight. Several specimens may be obtained through a single novocainized skin puncture. The reliability of the sampling can be verified by micro nitrogen and micro phosphate determinations. The biopsy is not unduly traumatizing and patients readily consent to its repetition. Biopsies of the gluteus maximus appear to be less painful than those made elsewhere.

III. THE THIAMIN CONTENT OF MICRO MUSCLE BIOPSIES

The yeast fermentation method devised by Schultz, Atkin and Frey[8] is admirably suited to the determination of thiamin in skeletal muscle. Pyrimidine blanks in this tissue are small, consistent, probably in fact negligible. The micro method[9] is readily converted to a lower range, Fig. 2, by utilizing standard 15

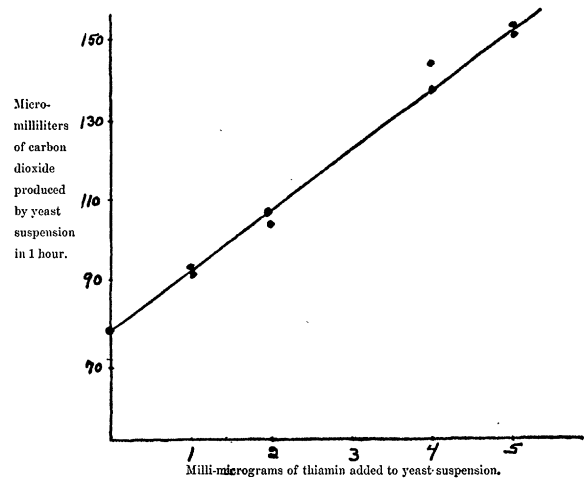

FIG. 2. Effect of thiamin, in 1 to 5 milli-microgram range, on carbon dioxide production of yeast suspension. (15 milliliter Warburg flask, 1 milliliter of nutrient medium, 1 milligram of yeast and 1 milliliter of thiamin standard.)

milliliter flasks fitted with a side-arm gas vent. This modification is sufficient to permit determination of thiamin in micro muscle biopsies secured with the Silverman needle.

Five to 15 mgs of muscle are transferred from the inner split needle to a small weighed tube. The muscle is ground, suspended in 0.02 M acetate buffer, (pH 4.8), and extracted. One milliliter of extract-suspension is pipetted into a 15 milliliter Warburg flask containing 1 mg of yeast in 1 milliliter of

[3] J. W. Ferrebee, N. Weissman, D. Parker and P. S. Owen, *Jour. Clin. Invest.*, 21: 401, July, 1942.

[4] J. W. Ferrebee, N. Weissman, D. Parker and P. S. Owen, "The Thiamin Content of Human Tissue." Association for Research in Nervous and Mental Disease, New York City, December 19, 1941. In press.

[5] This article: II. Micro Muscle Biopsies.

[6] This article: III. The Thiamin Content of Micro Muscle Biopsies.

[7] I. Silverman, *Am. Jour. Surg.*, 40: 671, 1938.

[8] A. S. Schultz, L. Atkin and C. N. Frey, *Jour. Indust. and Eng. Chem.*, (*Anal. ed.*), 14: 35, 1942.

[9] L. Atkin, A. S. Schultz and C. N. Frey, *Jour. Biol. Chem.*, 129: 471, 1939.

medium. The subsequent determination is carried out as described by Atkin, Schultz and Frey.[9]

Micro analyses[9] and analyses by this technique are in good agreement, Table IV. Novocain and adrenalin

TABLE IV

COMPARISON OF MICRO (8) AND SUBMICRO TECHNIQUES OF THIAMIN ANALYSIS. THIAMIN EXPRESSED AS MICROGRAMS PER GRAM TISSUE*

Cat	Date	Condition	Muscle thiamin	
			Micro analysis	Submicro analysis
Old white number 1	5/15	Normal	0.4	
		Heart 1.8, liver 2.3, kidney 2.2, Brain 1.4	0.4	..
			0.4	
Old gray number 2	6/22	Normal	..	0.5
				0.5
Old gray number 2	7/31	3 weeks semi-starvation, moderately deficient diet. Heart 1.0, liver 2.0, kidney 1.7, Brain 1.2	0.3	0.4
				0.4
			0.3	0.3
Young black number 3	6/23	Normal	..	0.7
				0.7
				0.8
Young black number 3	7/13	2 weeks semi-starvation, moderately deficient diet	0.3	0.3
			0.3	0.4
			0.3	0.3
			0.3	0.3
				0.3
				0.3
Young black number 3	7/23	3½ weeks semi-starvation, moderately deficient diet	0.22	0.27
			0.23	0.27
			0.22	0.25
			0.23	0.26
				0.25
				0.26
Young black number 3	7/27	2 days after subcutaneous injection of 2 mg. of thiamin	0.96	1.20
			0.99	1.15
			1.08	1.20
			1.08	1.10
				1.18

* For micro analyses 1 to 2 grams of skeletal muscle were removed surgically under nembutal anesthesia; for submicro analyses 5 to 15 milligrams of muscle were removed with the Silverman needle.

used in skin anesthesia do not interfere with measurements by the yeast fermentation method. When the muscle is abnormal, aliquots of suspension may be used for micro nitrogen or phosphorus determinations[10] and the thiamin concentration expressed in micrograms per milligram of muscle nitrogen or phosphorus.

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VARIABILITY IN THE PAIN THRESHOLD

HARDY, Wolff, Goodell and Schumacher have reported an unusual series of observations on the absolute pain threshold in man. Using radiant heat as a stimulus, they found only slight variability in pain thresholds, either for repeated measurements made upon the same person,[1] or for measurements made upon different subjects.[2] Furthermore, this threshold pain was said to be so uniform in quality that it was easily recognized even by untrained subjects. These authors attribute the commonly observed differences in pain sensitivity to conditions governing "reaction" to pain, rather than to fundamental differences in perceptual sensitivity as such.

In order to test the generality of the conclusion that pain thresholds are uniform, the writer has used electric current in a series of pain threshold measurements made upon 15 college women. An electronic device of the type recently described by Fender[3] supplied the current. This instrument produces condenser discharges which are amplified and delivered through resistance of such high order that variations in the subject's skin resistance have little effect upon the current flowing in the stimulus circuit. An A.C. microammeter measures this current directly. Current strength may be varied continuously by changing the resistances in series with the subject. The electrodes consisted of a silver disc 17 mm in diameter and a rounded silver wire 1 mm in diameter, embedded 8 mm apart in a piece of bakelite.

Threshold determinations were made on four skin areas, two each on the dorsal surface of the left forearm and on the forehead, in the following order: arm, head, head, arm. The "method of minimal changes" was used, with two "ascending" and two "descending" series for each spot. On a second day the experiment was repeated with 14 of the 15 subjects.

The mean of 240 threshold determinations made on the first day—irrespective of subject or of skin area—was 15.96 microamperes. The range of the thresholds was from 2.25 to 65 microamperes, while the standard deviation was 8.78 microamperes. If these variability indices are converted into relative units, the range represents a variation about the mean of approximately −80 to +300 per cent., while the standard deviation is ± 55 per cent. of the mean. The repetition of the experiment yielded slightly higher figures for the mean and standard deviation (18.18 ± 10.14), but the relative variability remained almost unchanged (S.D./Mean = 56 per cent.).

The variability of these pain threshold measurements is markedly greater than that reported by Hardy and his collaborators for thermal stimuli. Their standard deviation represented a variation

[10] Nessler and Kuttner-Lichtenstein techniques modified (see O. Schales, R. V. Ebert and E. A. Stead, Jr., *Proc. Soc. Exp. Biol. and Med.*, 49: 1, 1942; T. D. Fontaine, *Jour. Indust. and Eng. Chem.* (*Anal. ed.*), 14: 77, 1942) and adapted to Coleman spectrophotometer.

[1] J. D. Hardy, H. G. Wolff and H. Goodell, *Jour. Clin. Invest.*, 19: 649, July, 1940.

[2] G. A. Schumacher, H. Goodell, J. D. Hardy and H. G. Wolff, SCIENCE, n. s., 92: 110, August 2, 1940.

[3] F. A. Fender, SCIENCE, n. s., 89: 491, May 26, 1939.