



REFRACTOMETRIC DETERMINATION OF TOTAL SERUM PROTEIN:

Tawat Tositarat, B.Sc. (Med. Tech.) **

Kruawan Visuthisak, B.Sc. (Med. Tech.) *

Panja Kulapongs, M.D., Dip. Amer. Bd. Ped. ***

Abstract

A rapid and reliable estimation of total serum protein content can be made from the refractometric measurement using TS meter. Our experience indicated that the calibration standard provided by the manufacturer gives too low results compared to the standard biuret method. The new calibration scale is constructed for use in our lab. For practical purpose in nutritional field survey one can interpret the TS value (in gm./Lit.) as a total serum protein content (in gm./100 ml.)

INTRODUCTION.

The utilization of the physical property of refractive index of a fluid to quantitate its content is common in industry. (1) When applying this theory in human serum one can obtain accurate and rapid estimates of the content of total serum solids (2) and total serum proteins. (1, 3, 4) Rubini and Wolf has demonstrated the accuracy of refractometric determination of total solids in serum with a protein content range of 1.77 to 13.9 gm/

100 ml. and A/G ratio of 0.13 to 14.0. The estimation of the protein concentration of plasma or serum by refractometry has also been advocated for many year. (3,4,5) Although it is not as reliable as the measurement of total solids and specific gravity the accuracy of this technic in determination of total serum protein is satisfactory for clinical use. Extensive experimentation by many led to the improvement of technic and development of the widely used

* Technologist, Hematology Lab.

** Chief Technologist, Hematology Lab., St. Louis/Chiang Mai Research Center, Chiang Mai University.

*** Hematologist, Dept. of Pediatrics, Faculty of Medicine, Chiang Mai University.

TS meter. The TS meter is a Goldberg refractometer which has been designed specifically for medical use. The instrument is temperature compensated for temperature between 60° F. and 100° F.

From our recent field survey the wide discrepancy between the total serum proteins values obtained from the TS meter readings and those from biuret method. (6) It is the purpose to report our findings with the proposal of the newer TS meter calibration scale for use in our country.

Material and Method

The best group of patients who can provide a wider range of total protein content is children with protein calorie malnutrition were studied. Plasma samples were collected from microhematocrit (hepa-

rinized) tubes and the TSS value were read by the Goldberg TS meter. At the same time the venous blood total serum protein content was measured by the biuret method. (6)

Results

From the results shown in Table I, II and Figures I, II it is evident that there is a good correlation between the TS readings and total serum protein values (biuret method). The TS reading can be directly interpreted as the total protein content. The calibration scale provided by the manufacturer gives too low total protein values.

Hydration state of the patients exerts only minimal effect, if ever, to those correlation.

TABLE I. CORRELATION BETWEEN THE TSS READINGS AND TSP (Kjeldhal)
VALUES IN DIFFERENT FORMS OF MALNUTRITION

MARASMIUS			MARASMIC-KWASHIORKOR			KWASHIORKOR		
Case No.	TSS	TSP	Case No.	TSS	TSP	Case No.	TSS	TSP
4	5.2	6.05	1	5.2	3.75	2	4.2	3.50
8	6.4	6.60	5	5.1	5.90	3	3.5	2.69
10	8.0	7.98	7	4.6	4.90	6	3.8	4.20
11	6.8	7.10	9	7.2	7.60	14	4.0	4.30
13	7.0	8.4	12	6.0	6.40	22	3.3	3.66
15	6.1	5.8	17	4.6	5.50	24	3.4	3.80
16	5.7	7.5	18	6.2	6.70	25	3.6	4.00
19	6.6	6.7	20	5.4	5.80	30	3.8	4.30
26	5.8	5.8	21	5.6	5.90	31	4.4	5.00
27	5.1	5.3	23	5.2	4.88	33	4.4	4.40
28	7.5	7.9	29	5.1	5.10	51	3.0	3.66
32	7.8	8.8	35	5.8	5.3	52	3.5	3.56
34	6.5	6.9	36	3.8	4.5	59	4.3	4.38
37	5.6	6.10	40	4.1	4.8	61	3.5	3.46
38	5.6	5.70	41	5.4	5.3	62	3.5	3.54
39	6.8	6.80	42	5.5	4.6	63	3.3	3.75
43	7.6	7.40	45	4.0	4.58	64	3.5	3.80
44	4.6	4.46	46	3.8	3.98	65	3.4	3.40
47	7.1	7.30	53	5.6	6.10	66	4.3	4.48
48	6.4	6.40	55	4.1	3.98	74	3.9	4.17
49	4.1	4.10	56	5.2	4.70			
50	6.5	7.50	57	4.1	4.24			
54	5.8	5.00	60	4.1	4.44			
58	5.5	6.00	67	5.3	5.50			
68	6.0	5.60	70	5.1	4.90			

MARASMUS			MARASMIC-KWASHIORKOR			KWASHIORKOR		
Case No.	TSS	TSP	Case No.	TSS	TSP	Case No.	TSS	TSP
69	5.3	5.20	73	4.5	4.24			
71	6.5	7.14	76	6.1	4.8			
72	6.5	6.90	77	5.7	5.5			
75	7.0	6.46						
78	6.6	6.35						

NOTE: See Figure I.

TABLE II. EFFECT OF HYDRATION ON REFRACTOMETRIC READINGS
(TSS) AND TSP VALUES

Case No.	BEFORE HYDRATION		AFTER HYDRATION		
	TSS	TSP	TSS	TSP	
32	7.8	8.8	6.6	7.0	
33	4.4	4.4	4.5	4.7	
34	6.5	6.9	6.5	6.0	
35	5.8	5.3	5.7	6.1	
36	3.8	4.5	4.7	5.1	
37	5.6	6.1	6.0	6.8	
38	5.6	5.7	6.7	7.1	
39	6.8	6.8	5.5	6.2	
40	4.1	4.8	4.6	5.4	
41	5.4	5.3	5.2	5.3	
42	5.5	4.6	4.5	4.67	
43	7.6	7.4	6.3	6.9	
44	4.6	4.46	4.2	4.62	
45	4.0	4.58	4.4	4.4	
46	3.8	3.98	4.4	3.99	

Case No.	BEFORE HYDRATION		AFTER HYDRATION		
	TSS	TSP	TSS	TSP	
47	7.1	7.3	7.2	6.8	
49	4.1	4.1	4.2	4.5	
50	6.5	7.5	6.5	7.1	
51	3.0	3.66	3.8	3.93	
52	3.5	3.56	4.5	4.0	
53	6.7	6.25	5.6	6.1	
54	5.8	5.0	6.5	5.68	
55	4.1	3.98	3.6	3.48	
56	5.2	4.7	4.5	4.92	
57	4.1	4.24	4.8	4.54	
58	5.5	6.0	5.5	5.65	
59	4.3	4.38	5.1	5.15	
60	4.1	4.44	4.1	4.16	
61	3.5	3.46	5.1	4.9	
62	3.5	3.54	3.7	3.54	
63	3.3	3.75	4.0	4.0	
64	3.5	3.8	3.8	3.8	
65	3.4	3.4	3.8	3.43	
66	4.3	4.48	4.5	4.38	
67	5.3	5.5	5.0	5.2	
68	6.0	5.6	5.1	5.4	
69	5.3	5.2	5.3	5.1	
71	6.5	7.14	6.0	6.05	
73	4.5	4.24	3.9	4.48	
77	5.7	5.5	6.0	5.75	

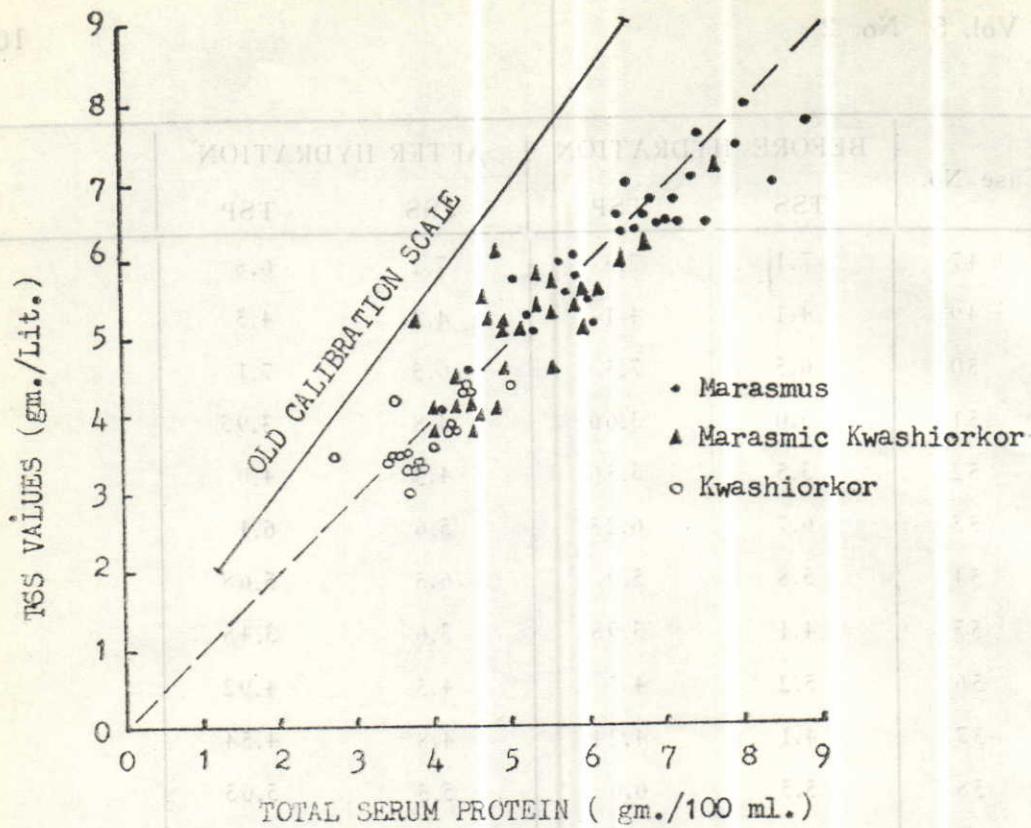


FIGURE I. CORRELATION BETWEEN THE TSS AND TSP VALUE

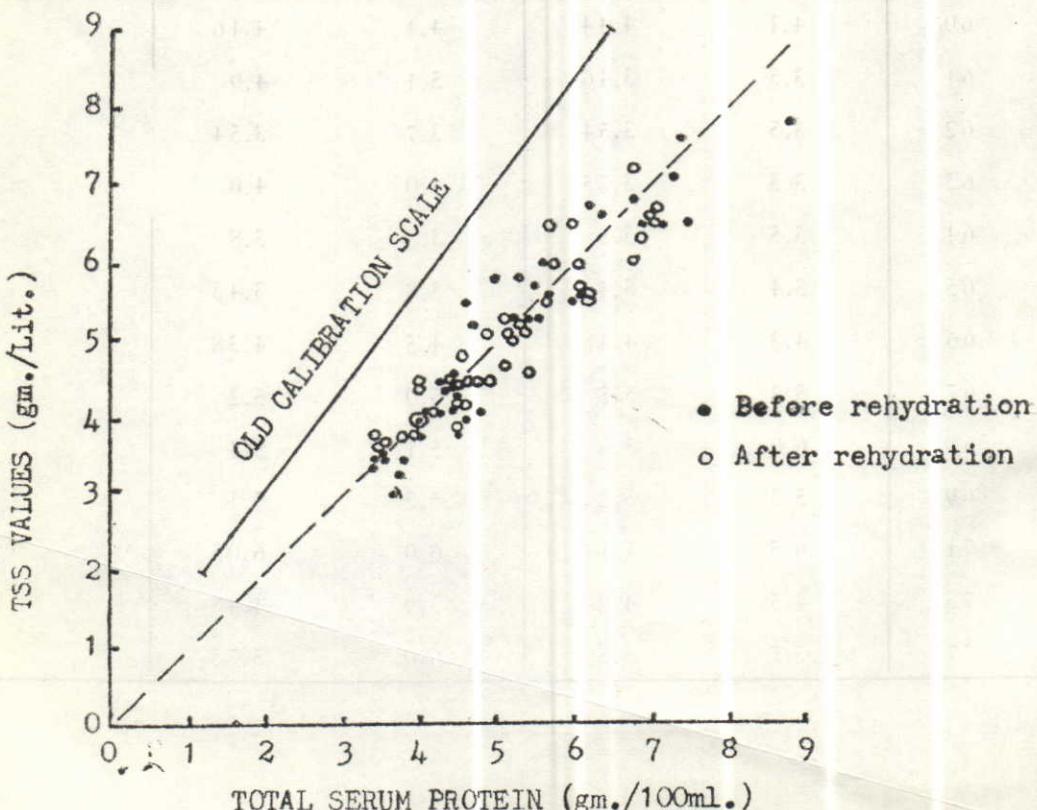


FIGURE II. EFFECT OF REHYDRATION ON THE CORRELATION BETWEEN

COMMENTS.

The use of refractive index changes to measure serum protein concentration has been established as a most rapid and useful technic for many years. (3, 7) and it is recently being adapted for auto-analytic procedure. (7) It can be expected that refractometry will allow a greater variability of result in the determination of total serum protein than does the biuret method. The early reports on the use of refractive index for serum proteins determination stated that a correction factor must be applied for the non-protein constituents of serum. (1, 7) These components include electrolytes, glucose, urea, bilirubin, cholesterol and lipids. Marsh and Fingerhut recently demonstrated that glucose value up to 400 mg./ml. and urea N. values up to 200 mg./100 ml. caused changes of the refractive index which were within the tolerance. Bilirubin and turbidity either artificially produced or present as a result

of pathological conditions, caused virtually no effect. (7, 8) The exact effect of very high cholesterol or lipid level was difficult to assess since artificially prepared solutions alter the protein nature of the test solution. (7) The validity of this method in the estimation of total serum proteins in specimens with varying albumin and globulin content and A/G ratios has been questioned but the recent study by Barry and associates indicated that its accuracy is entirely satisfactory for clinical use. Our experience also indicated degree of dehydration, albumin, and globulin content, jaundice and turbidity has no significant effect on the TS refractometry.

For practical purpose of nutritional survey it is suggested that we may adopt the TS reading directly as the total serum proteins in grams/100 ml. The confirmation of above findings is still required from other laboratories.

REFERENCE

1. Neuhausen, B.J., and Rioch, D.M.: The refractometric determination of serum proteins, *J. Biol. Chem.* 55:353, 1923.
2. Rubini, M.E., and Wolf, A.V.: Refractometric determination of total solids and water of serum and urine, *J. Biol. Chem.* 225:869, 1957.
3. Sunderman, F.W.: A rapid method for estimating serum proteins: Formula for calculating serum protein concentration from the refractive index of serum. *J. Biol. Chem.* 153:139, 1944.
4. Sunderman, F.W., and Boerner, R.: *Normal Values in Clinical Medicine*. Phila., 1949.
5. Gettler, A.O., and Baker, W.: *J. Biol. Chem.* 25:211, 1916.
6. Kingsley, G.R.J. *Lab. Clin. Med.* 27:840, 1942.
7. Marsh, W.H., and Fingerhut, B.: Serum protein determination by automatic recording refractometry. *Clin. Chem.* 8:640, 1962.
8. Barry, K.G., McLaurin, A.W., and Parnell, B.L.: A practical temperature compensated hand refractometer (the TS meter): Its Clinical used and application in estimation of total serum proteins. *J. Lab. Clin. Med.* 55:803, 1960.