Age of horse	Percentage of total protein				Protein conc.
	Albumin	($\alpha_1+\alpha_2$) glob.	($\beta_1+\beta_2$) glob.	γ glob.	
Newly born	65	32	3	0	3·7
After 5 days suckling	37	36	25	2	5·25
Foal, 8 months	33	14	25	28	6·8
2½ years	30	11	33	26	7·0
10 years	30	11	12	47	7·0

ance of traces of γ globulins. With age the albumins decrease slightly, and the α globulins markedly, to a constant. The β and γ globulins decrease and increase respectively in marked degree so that the total protein concentration remains constant at a figure slightly less than double that of the newly-born foal.

Jameson, Alvarez-Tostado and Sortor[4] reported results on the electrophoresis of calf serum. They showed that the increase in γ globulin is very rapid during the nursing period; in other respects their results resemble the results reported in this communication.

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[1] Polson, NATURE, **145**, 27 (1940).
[2] Tiselius, *Biochem. J.*, **31**, 313 (1937).
[3] Lamm, *Z. Phys. Chem.*, **138**, 313 (1928).
[4] Jameson, Alvarez-Tostado and Sortor, *Proc. Soc. Exp. Biol. Med.*, **51**, 163 (1942).

A New Source of Trehalose

ABOUT fifteen years ago the manna of the Sinai desert was identified by Bodenheimer and Theodor[1] as the excretion of the scales *Trabutina mannipara* and *Najacoccus serpentinus* on the leaves of *Tamarix mannifera*. Chemically, the manna was determined as a mixture of sucrose and invert sugar[2].

Recently, Prof. Bodenheimer kindly supplied me with specimens of a manna from the North Iraqian desert (Suleimanyia District). The Bedouin gather this sweet product from leaves of trees and bushes, and use it as a sugar substitute in coffee. It is a half syrupy, half crystalline mass, and is considered by Prof. Bodenheimer to be an excretion of a leaf scale. Chemical analysis showed that its sugar fraction consists mainly of the rare disaccharide trehalose, which could be isolated from the mixture after elimination of the fermentable sugar by yeast, deproteinization, concentration and extraction of the residue with boiling alcohol. It showed the correct optical activity: $[\alpha]_D$ + 198°. One manna specimen contained 30 per cent and the other 45 per cent of trehalose calculated on the total dry matter, and 70 per cent and 80 per cent of trehalose calculated on the total carbohydrate content. The remaining carbohydrate consisted of sucrose and invert sugar containing an excess of glucose.

Previously, trehalose has been shown to occur in another rare manna (trehala-manna), which has been identified as the cocoon of an oriental beetle, and in yeast, other fungi, and species of Selaginella[3].

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[1] "Ergebnisse der Sinai-Expedition 1927 der Hebräischen Universität", p. 45 (Leipzig, 1929).
[2] Fodor and Cohn, Ibid., p. 89.
[3] Zemplen, "Biochemisches Handlexikon", **13** (Suppl. Vol. 6), 551 (1931).

Microbiological Assay of Riboflavin

IN 1939, Evans, Handley and Happold[1] used the Strong-Snell[2] method to demonstrate the synthesis of riboflavin, among other elements of the B complex, by *C. diphtheriæ*, and early in 1940 I demonstrated to the Biochemical Society the Strong-Snell technique for the assay of this component. In further studies we became aware of certain pitfalls in the application of this method; our observations being communicated to fellow investigators at a symposium held in Reading a year ago.

Since then full papers on the microbiological assay of riboflavin by Barton-Wright and Booth[3] and by F. W. Chattaway and Mary Sandford and myself[4] have appeared. The formers' paper, which appeared first, does not mention at least two facts of importance which must be considered before the original method of Strong and Snell can be applied, namely, the importance of the calcium level of the medium, and of the additional growth factors which must be present in the medium in optimal proportions. Both of these points have been conceded in private conversation. It is therefore to be regretted that in their recent communication to NATURE[5] which appears simultaneously with that of Prof. Hopkins[6] there is no mention of these particulars. It seems a pity that we should run the risk of discrediting a potentially valuable line of approach to the rapid assay of the vitamins of the B complex by failing to indicate to all workers in this field the necessity for careful and critical scrutiny of the methods in use before we apply them in a wholesale fashion to the analysis of foods and body fluids and secretions.

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Leeds, 2.
Sept. 14.

[1] Evans, W. C., Handley, W. R. C., and Happold, F. C., *Brit. J. Exp. Path. and Bact.*, **20**, 396 (1939).
[2] Snell, E. E., and Strong, F. M., *Ind. Eng. Chem.* (Anal. ed.), **11**, 346 (1939).
[3] Barton-Wright, E. C., and Booth, R. G., *Biochem. J.*, **37**, 25 (1943).
[4] Chattaway, F. W., Happold, F. C., and Mary Sandford, *Biochem. J.*, **37**, 298 (1943).
[5] Barton-Wright, E. C., Moran, T., and Sarson, H. S., NATURE, **152**, 273 (1943).
[6] Hopkins, R. H., NATURE, **152**, 274 (1943).

DR. HAPPOLD has shown us a copy of his letter criticizing the microbiological assay of riboflavin by our modification of the Snell and Strong[1] method. Had his criticisms been raised against the assay of pantothenic acid using *Lactobacillus helveticus* or nicotinic acid using *L. arabinosus* (Snell and Wright[2]) we would have been in partial agreement, but as regards riboflavin assay they have no justification.

Further, we would point out that our paper was submitted for publication a fortnight before the Reading symposium (held September 25, 1942), while we certainly have never conceded in private conversation the point that Dr. Happold raises.

The Snell and Strong medium uses photolysed peptone and yeast extract for the necessary remaining factors other than riboflavin required in optimum amount. The main reason for the success of the riboflavin assay by this method and lack of success with some other members of the B_2 complex is due to the fact that the riboflavin is easily removed from peptone, and yeast without destroying the other factors. Peptone itself contains an unknown factor