[CONTRIBUTION FROM THE RESEARCH CHEMICAL LABORATORY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION]

THE VAN SLYKE METHOD FOR PROTEIN ANALYSIS AS AFFECTED BY FATS[1]

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The Van Slyke method[2] for protein analysis was designed for the differentiation of pure proteins by the determination of chemical groups characteristic of certain amino acids. Wishing to investigate the distribution of amino acids in naturally occurring protein materials, numerous investigators attempted to use this method for the determination of the nitrogen distribution of various materials containing not only proteins but carbohydrates, fibers, fats and various other constituents present in plant and animal substances. The confusion arising from this work led Van Slyke[3] in 1915 to call attention again to the fact that his method was devised to be used with pure proteins, not accompanied by other substances.

The effect of carbohydrates and fiber on the Van Slyke method has been thoroughly investigated, especially by Gortner and his co-workers.[4] They have shown that these substances materially affect the nitrogen distribution of this method and have ascribed the effect to the action of the aldehyde group.

Although considerable work has been done on the effect of carbohydrates, a survey of the literature has failed to disclose any similar work having been conducted on the effect of fats and related compounds on the Van Slyke method. However, Morrow[5] stated: "The Van Slyke method of analysis for the determination of certain amino acids is limited in its application to pure proteins, to solutions of practically pure proteins, or to protein substances comparatively free from carbohydrates, fibers, fats, etc."

It therefore seemed desirable to make a critical study of the effect of the presence of fat and related compounds on the nitrogen distribution of proteins as determined by the Van Slyke method.

[1] Presented before the Division of Agricultural and Food Chemistry at the Indianapolis Meeting of the American Chemical Society, March 31, 1931. Published with the approval of the Director of the Purdue University Agricultural Experiment Station.

[2] D. D. Van Slyke, *J. Biol. Chem.*, **10**, 15–55 (1911).

[3] D. D. Van Slyke, *ibid.*, **22**, 281–285 (1915).

[4] (a) Gortner and Blish, THIS JOURNAL, **37**, 1630–1636 (1915); (b) Gortner and Holm, *ibid.*, **39**, 2477–2501 (1917); (c) Gortner, *J. Biol. Chem.*, **26**, 177–204 (1916); (d) Holm and Gortner, THIS JOURNAL, **42**, 632–640 (1920).

[5] C. A. Morrow, "Biochemical Laboratory Methods," John Wiley and Sons, Inc., New York, **1927**, p. 161.

Experimental

Throughout this investigation, the technique for the Van Slyke method as summarized in Morrow's text, "Biochemical Laboratory Methods,"[5] was used. A description of the materials hydrolyzed as well as the results of the analyses are given in tables. The figures for each individual treatment represent the average of two, three or more determinations made from individual hydrolyses.

In the first series of experiments, the protein used was casein (Hammersten). Three grams of casein was hydrolyzed alone; in the presence of butter fat; in the presence of individual representative constituents of fat, such as glycerol, stearic acid, representing the saturated group of fatty acids, and oleic acid representing the unsaturated groups; and, finally, in presence of carnauba wax, which is not a triglyceride. The amounts of materials used and the results of the analyses obtained from these hydrolysates are given in Table I.

TABLE I

THE EFFECT OF THE PRESENCE OF FAT, GLYCEROL, STEARIC ACID, OLEIC ACID AND CARNAUBA WAX ON THE VAN SLYKE ANALYSIS OF CASEIN (AVERAGE RESULTS)

Material hydrolyzed, 3 g. of casein +	Ammonia nitrogen, mg.	Humin nitrogen			Basic nitrogen			Non-basic nitrogen		Nitrogen recovered, mg.
		Acid insol., mg.	Acid sol., mg.	P. T. A., mg.	Amino, mg.	Non-amino, mg.	Arginine, mg.	Amino, mg.	Non-amino, mg.	
	41.02	3.01	6.65	2.10	66.76	35.16	33.42	243.15	16.95	414.80
2 g. butter fat	41.42	2.59	10.34	3.54	61.62	36.64	29.96	230.72	20.28	407.15
2 g. glycerol	41.23	2.48	10.36	4.87	58.55	43.72	24.52	186.10	58.82	406.13
3 g. glycerol	41.46	2.79	19.39	5.60	54.95	53.43	21.68	174.66	60.54	412.82
3 g. stearic acid	41.20	2.73	6.58	2.14	68.92	35.24	33.08	240.96	13.84	411.61
3 g. oleic acid	41.37	3.23	6.36	2.00	66.38	34.42	33.32	237.42	15.92	407.10
3 g. carnauba wax	41.20	2.57	5.32	2.10	64.29	33.71	30.80	234.10	19.40	402.69

It will be noted that the ammonia nitrogen remained about constant in all cases. This was true also of the acid-insoluble humin. In the samples hydrolyzed in the presence of butter fat, there was an increase in the acid-soluble humin, the phosphotungstic acid humin and a decrease in the amino nitrogen of both the basic and non-basic fractions. As a consequence of the decrease in amino nitrogen, there is an increase in the non-amino nitrogen of these fractions because these values are obtained by the difference between the total nitrogen and the amino nitrogen. There is also a significant decrease in the arginine nitrogen value. The values for the other amino acids found in the basic nitrogen fraction are omitted because cystine is present only in small amounts and any values calculated for histidine and lysine which were based on such decreased amino and arginine values as obtained in the presence of fat, would be of no significance.

Since the Van Slyke method was found to be affected by fat, the next step

was to determine the constituent responsible for this action. In the tests with glycerol, stearic acid and oleic acid, it was found that the fatty acids were without effect, while glycerol affected the same groups that were affected by the butter fat but to a greater extent. Thus it appears that glycerol is the reactive agent responsible for the effects produced.

The tests with carnauba wax were conducted for the purpose of determining the effect of wax, and especially of a material which is not a triglyceride, upon the Van Slyke method. The results show that carnauba wax had little, if any, effect on the nitrogen distribution.

These effects produced by fats and glycerol on the results of the Van Slyke analyses are in some respects similar and in others unlike that produced by carbohydrate material. Unlike the action of carbohydrates, glycerol or fats do not cause the formation of acid-insoluble humin, but in respect to the other fractions, the effects are similar. Gortner[4c] observed that in addition to the formation of acid-insoluble humin, carbohydrate material caused an increase in the acid-soluble humin, phosphotungstic acid humin and non-amino nitrogen, and a decrease in the arginine nitrogen and amino nitrogen, similar to that obtained in these experiments with fat and glycerol.

In the second series of experiments, gelatin (Bacto-Gelatine, Difco Standardized) was used as the protein. Since gelatin contains little or no tyrosine, tryptophane, cystine and histidine, it was thought that analyses of this protein with and without additions of glycerol might give an insight into the amino acid involved in the formation of the acid-soluble and phosphotungstic acid humins, as well as show what effect the absence of a number of amino acids might have on the other fractions. The results are given in Table II. It is to be observed that the results are quite different from those obtained with casein. There is no increase in the acid-soluble humin and phosphotungstic acid humin nitrogen. However, the amino nitrogen and arginine fractions are decreased in a similar manner as before.

TABLE II

THE EFFECT OF THE PRESENCE OF GLYCEROL ON THE VAN SLYKE ANALYSIS OF GELATIN (AVERAGE RESULTS)

Material hydrolyzed	Ammonia nitrogen, mg.	Humin nitrogen: Acid insol. mg.	Humin nitrogen: Acid sol., mg.	Humin nitrogen: P.T.A., mg.	Basic nitrogen: Amino, mg.	Basic nitrogen: Non-amino, mg.	Basic nitrogen: Arginine, mg.	Non-basic nitrogen: Amino, mg.	Non-basic nitrogen: Non-amino, mg.	Nitrogen recovered, mg.
3 g. gelatin	18.18	0.29	1.48	5.38	110.80	61.53	67.76	209.21	53.37	459.24
3 g. gelatin + 3 g. glycerol	18.35	.31	1.38	5.20	80.90	88.90	56.4	190.22	71.48	455.74

The third series of experiments were performed to determine the effect of glycerol on arginine, tyrosine and tryptophane. These amino acids were Pfanstiehl products used without further purification. The results of these analyses are given in Table III. In the case of arginine, the ar-

ginine value and amino nitrogen were decreased by the presence of glycerol. Concerning the ammonia and humin nitrogen fractions, the differences were not large enough to be significant. For the unexpected presence of nitrogen in the non-basic fraction, no explanation is offered.

TABLE III

THE EFFECT OF GLYCEROL ON THE NITROGEN DISTRIBUTION OF SOME AMINO ACIDS (AVERAGE RESULTS)

Material hydrolyzed	Ammonia nitrogen, mg.	Humin nitrogen Acid insol., mg.	Humin nitrogen Acid sol., mg.	Humin nitrogen P.T.A., mg.	Basic nitrogen Amino, mg.	Basic nitrogen Non-amino, mg.	Basic nitrogen Arginine, mg.	Non-basic nitrogen Amino, mg.	Non-basic nitrogen Non amino, mg.	Nitrogen recovered, mg.
3 g. arginine	0.07	0.07	0.07	1.26	21.11	55.19	77.44	5.46	2.26	86.49
3 g. arginine + 3 g. glycerol	.14	.21	.21	1.94	17.28	57.76	61.04	3.98	4.67	86.19
2 g. tyrosine	.0	.0	.0					23.44	.0	23.44
2 g. tyrosine + 3 g. glycerol	.16	.0	4.52					14.30	4.22	23.20
3 g. tryptophane	.07	.70	0.42					20.69	19.35	41.21
3 g. tryptophane + 3 g. glycerol	.12	.80	1.12					17.95	22.26	42.25

The experiments with tyrosine clearly indicate that tyrosine hydrolyzed in the presence of glycerol tends to form acid-soluble humin. It is interesting to note that tyrosine has also been found to be responsible for the formation of acid-soluble humin when hydrolyzed in the presence of aldehydes.[4c] There was a definite decrease in amino nitrogen.

Unlike the action of aldehydes on tryptophane,[4c] glycerol does not cause the formation of acid-insoluble humin. However, there is some indication that it does contribute some nitrogen to the acid-soluble humin. Again, it will be observed that glycerol caused a decrease in amino nitrogen.

Summary

1. The Van Slyke method for protein analysis is affected by the presence of fat.

2. The reactive constituent of the fat molecule is glycerol; the fatty acids such as stearic acid, representing the saturated group, and oleic acid representing the unsaturated groups, are without effect.

3. The significant effects produced by fat or glycerol are: an increase in the acid-soluble humin, in the phosphotungstic acid humin, and in the non-amino nitrogen, and a decrease in the amino nitrogen and arginine values.

4. Carnauba wax, which is not a glyceride, had little, if any, effect on the Van Slyke method.

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