A Hitherto Undescribed Coloring Reaction of Certain Human Nerve Fibers

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This note describes a peculiar and consistent coloring reaction of certain human nerve fibers. No similar description has been found in the literature. The authors do not propose to investigate the matter further at the present time, but mention of the phenomenon may stimulate others to do so.

In brief, the new reaction is observed in frozen sections of fresh unfixed human tissues, colored with the periodic acid-Schiff's reagent (PAS) method (*4*). The optimum tissue for the study of this phenomenon is that of the nerve ganglia buried in fatty tissue anterior to the aorta, between the celiac axis and the superior mesenteric artery.

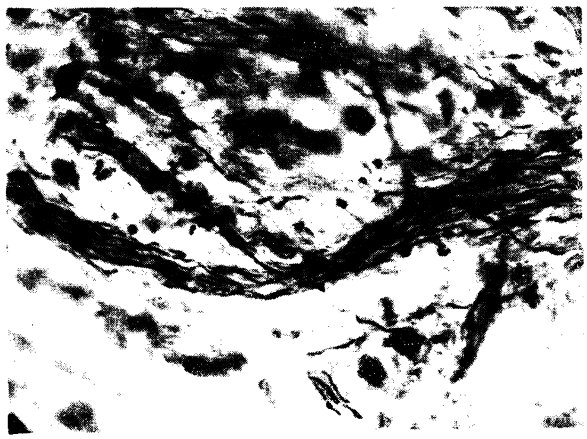

FIG. 1. Human preaortic ganglion, colored by the PAS method. Frozen section, unfixed tissue.

The ganglia, dissected free of fat, and colored with the PAS method on frozen sections, present the appearance shown in Fig. 1.

In such an area it can be seen that many nerve fibers are left uncolored, although some do color with the PAS method. The process of paraffin imbedding removes the faculty of selective coloration. The reaction is not that due to Schiff's reagent alone. Acidity (basophilia) of the fibers is not responsible. The age is not important, nor have we been able to correlate variation with any specific disease.

Relatively few other tissues have been examined for the peculiar reaction of the nerve fibers. In human pectoral muscle a few nerve fibers in the connective tissue give the positive reaction. The termination of these fibers has not been ascertained. In the broad ligament, single nerve fibers give the positive reaction. In these situations repeated observations show the reaction to be selective and consistent.

It is not possible to ascertain the nature of the material responsible for the selective coloration of certain nerve fibers. The fact of its demonstration with the PAS method, developed for carbohydrates (*3*), permits certain inferences. It has been shown that a mucoprotein with choline esterase activity can be isolated from human serum (*1*). The presence of choline esterase at nerve terminations has long been realized (*2*) but its origin is not known. It may be that a carbohydrate of choline esterase type is present in some nerve fibers and is being shown by the present method.

It is repeated that only human tissues have been used in this study and only as unfixed, frozen sections.

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Cumulative Frequency Distribution in Manual Chromatography[1]

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The applicability of partition chromatography to the measurement of physiologically important organic acids has been reported previously (*1*). The chromatographic technique has also been used in measurement of amino acids in protein hydrolyzates (*3*). During studies of the citric acid metabolic cycle in this laboratory, a system of manual chromatography was devised which obviates the tedious collection of each fraction of an effluent acid band. This procedure, unlike that involving collection of effluent acid without fractionation, provides qualitative evidence dependent upon the characteristics of the curve of the chromatogram. This report describes a procedure which increases the practicability of manual chromatography.

The procedure is based on the principle that a cumulative normal distribution, when plotted on arithmetic probability paper, gives a straight line (*4*). Application of this principle to chromatography makes unnecessary the collection of every sample within the predetermined acid zone. The quantities of acid in the fractions collected can be regarded as cumulative frequencies in a normal distribution (*2*). The sum of the quantities of acid in the fractions collected within a given acid zone is equal to the amount of that acid introduced on the column (*2*).

The data presented here were obtained by analyzing a mixture of succinic and fumaric acids. The procedure followed that previously reported for fumaric acid alone (*2*) except that columns of 4 mm instead of 8 mm internal diameter were employed, and 0.5 g rather than 3 g of silica gel was used in each column. Further, 10%

[1] Supported in part by a grant from the Damon Runyon Memorial Fund.

tertiary amyl alcohol-chloroform instead of 5% amyl alcohol was the mobile phase. The columns were 25–27 cm in height. Experience with this type of column and mobile phase had shown that fumaric acid data, conventionally plotted, describe a curve on the chromatogram which deviates from the normal. Succinic acid, however,

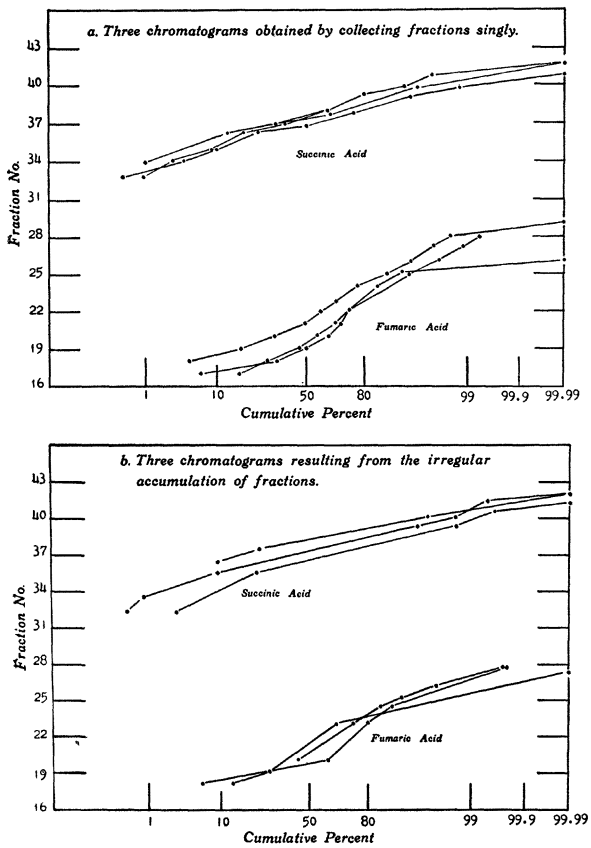

FIG. 1. Cumulative frequency graphs on probability paper of six chromatograms of a mixture of fumaric and succinic acids.

yields a normal curve. Thus, when a mixture of fumaric and succinic acids was examined, each effluent acid was released in a different way by the single column. This provided an ideal test for evaluation of cumulative collection. For example, repeated analysis indicated that when each fraction was collected singly the effluent fumaric acid appeared between chromatogram fractions 17 and 28, whereas succinic acid appeared between fractions 33 and 42. In one experiment, two groups of two or more fractions combined were collected within each of the fumaric and succinic acid zones. At least the first two and last two fractions of each acid were collected singly. In other experiments, the number of groups as well as the number of fractions forming the groups varied. The concentration of each single fraction and each group was determined by titration with standard alkali. The volume of alkali required for each single fraction and each group was considered the frequency statistic, and cumulative percentages were calculated (*4*).

Fig. 1 presents six chromatograms on probability paper. In Fig. 1a, three were obtained by collecting fractions singly. Three typical chromatograms resulting from the irregular accumulation of fractions are shown in Fig. 1b. The data for succinic acid in the experimental collections, as in the controls, approximate a straight line. The data for fumaric acid, because of the irregular release of this acid, as already explained, describe irregular lines in both the experimental and control measurements. A comparison of the position of the mode for succinic acid (i.e., the fraction number at the 50th percentile) and a comparison of the recovery data for both procedures are shown in Table 1.

TABLE 1

COMPARISON OF CUMULATIVE AND CONVENTIONAL METHODS FOR COLLECTING FRACTIONS WITHIN AN EFFLUENT CHROMATOGRAPHIC BAND

	Chromatogram No.	Fumaric acid			Succinic acid			
		Taken mg	Found mg	Recovery %	Taken mg	Found mg	Recovery %	Mode*
Conventional method	1	1.74	1.89	109	2.4	2.21	92	37.3
	2	1.74	1.80	103	2.4	2.19	91	37.5
	3	1.74	1.81	104	2.4	1.92	80	37.5
	Average	1.74	1.83	105	2.4	2.10	88	37.4
Cumulative method	4	1.74	1.90	109	2.4	2.29	95	38.0
	5	1.74	1.78	102	2.4	2.32	97	36.0
	6	1.74	1.91	110	2.4	2.40	100	37.0
	Average	1.74	1.86	107	2.4	2.30	97	37.0

* Fraction No. at 50th percentile. Because of the irregularity of the curve for fumaric acid (explained in text), the mode, being meaningless, is not presented for fumaric acid.

The final data for cumulative chromatography can be presented on probability paper with each curve representing an effluent acid. If this curve approximates a straight line there is evidence that the acid was released normally by the column. The irregular release of an acid by the column is reflected by a nonlinear curve. Thus collection of each fraction of an acid is obviated and random accumulation within the effluent band is adequate.

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