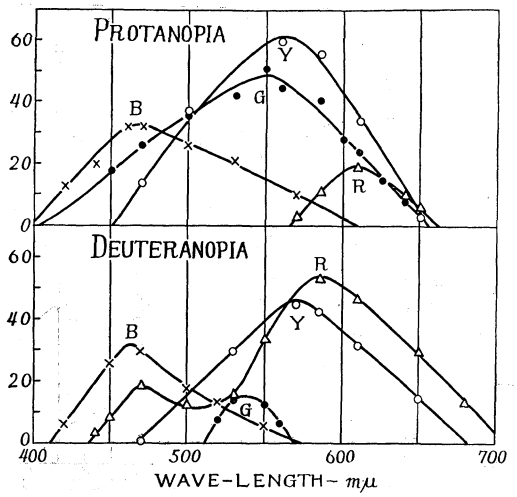

FIG. 2. Curves for spectral distribution of 4 retinal processes of dichromats.

shows that the distribution curves for the deteriorated processes, viz., the *R* in the protanope and the *G* in the deuteranope, are much lower and narrower than the other curves.

The well-known theory of Young assumes that one of three components is lacking in dichromats, but this claim obviously does not conform to our findings, (1) because there are, in reality, four components instead of three; and (2) because no component is entirely lacking, although the *R* or *G* component, in the respective color-blind types, is weaker than any others. It is to be expected from Young's theory that the sensitivity to yellow of dichromats would be deteriorated on account of the absence of the *R* or *G*, but in reality no such serious deterioration as would be expected from the theory can be demonstrated. Moreover, in rare cases such as those reported by v. Kries (*3*) and by Judd (*4*), in which one eye was color-

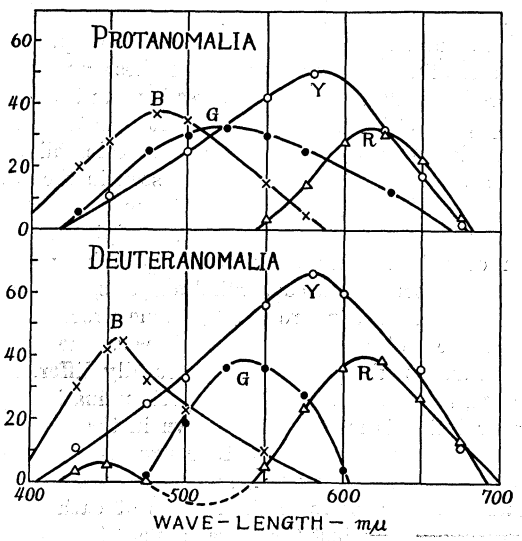

FIG. 3. Curves for spectral distribution of 4 retinal processes of anomalous trichromats.

anomalous and the other was normal, the part of the spectrum which looked yellow to the normal eye also looked yellow or yellowish to the anomalous eye. These facts which the trichromatic theory fails to interpret can easily be understood on the basis of our finding that the *Y* component is well developed in the fovea of dichromats. It is generally believed that there is a qualitative difference between anomalous trichromatism and dichromatism. In the light of our findings the difference is, however, merely quantitative (Fig. 3).

References

1. MOTOKAWA, K. *J. Neurophysiol.*, **12**, 291 (1949).
2. ———. *Ibid.*, 465.
3. VON KRIES, J. *Z. Sinnesphysiol.*, **50**, 137 (1919).
4. JUDD, D. B. *J. Research*, **41**, 247 (1948).

Chromatographic Separation of Amino Sugars and Amino Acids, Using the N-(2:4-dinitrophenyl) Derivatives

P. W. Kent,[1] G. Lawson, and A. Senior

A. E. Hills Laboratories,
University of Birmingham, England

In the study of bacterial polysaccharides and mucocomplexes, the need has been found for more sensitive methods of detection and estimation of microquantities of amino sugars in the presence of amino acids, neutral sugars, and uronic acids.

The ordinary procedures of paper chromatography (*1*), investigated independently (*2*), were found to be suitable for the separation of mixtures of neutral partly methylated sugars. The amino sugars, studied by this method, separated much less readily, because of a slow rate of migration and the diffuse character of the spots—since thought to be due to the ion-exchange behavior of the papers. Some reducing amino sugars could only be observed by their reaction with alkaline silver nitrate or with ninhydrin, whereas others, mainly nonreducing amino sugar derivatives—e.g., glucosides—could only be detected by spraying the chromatogram with dilute alkaline potassium permanganate (*3*). The introduction of aniline hydrogen phthalate (*4*) as an improved general reaction for reducing sugars gave little or no coloration with free amino sugars or N-acetylated sugars. It has now been found that N-(2:4-dinitrophenyl) reducing amino sugars (*5*) did react when warmed with aniline hydrogen phthalate.

Investigation showed that amino sugars would react readily under mild conditions (viz., room temperature in the presence of sodium bicarbonate) with 2:4-dinitrofluorobenzene forming stable N-(2:4-dinitrophenyl) derivatives and that the colored nature of these compounds rendered them preeminently suitable for chromatographic analysis. A mixture of neutral sugars, a uronic acid, and DNP[2] glucosamine was

[1] I.C.I. fellow of Birmingham University.
[2] DNP = N-(2 :4-dinitrophenyl).

TABLE 1

R_F VALUES

(40% butanol—10% ethanol—50% water [neutral] Whatman No. 1 filter paper downward migration)

	R_F	Silver Nitrate	Aniline Hydrogen Phthalate	Ninhydrin
3:4-dimethyl-D-mannose	0.53	+	+	−
3:4:6-trimethyl-D-mannose	.70	+	+	−
2:3:4:6-tetramethyl-D-mannose	.68	+	+	−
2:3-dimethyl-D-glucose	.51	+	+	−
2:3:4-trimethyl-D-glucose	.70	+	+	−
2:3:6-trimethyl-D-glucose	.66	+	+	−
2:3:4:6-tetramethyl-D-glucose	.85	+	+	−
1:3:4-trimethyl-D-fructose	.67	+	−	−
1:3:4:6-tetramethyl-D-fructose	.75	+	−	−
D-glucosamine hydrochloride	.1	+	−	+
N-acetyl-D-glucosamine	.24	+	−	+
N-carbobenzoxy α methyl glucosaminide	.82	−	−	−
3-methyl N-acetyl glucosamine	.34	+	−	−
4:6-dimethyl N-acetyl glucosamine	.50	+	+	−
3:4:6 trimethyl N-acetyl glucosamine	.72	+	+	−
glucosaminic acid	.02	−	−	+
α methyl 2(acetamido-) glucopyranoside	.31	−	−	+
β methyl 2-(acetamido-) glucofuranoside	.42	±	±	−
N-(2: 4 dinitrophenyl) D-glucosamine	.75	+	+	−
1:3:4:6 tetracetyl DNP-D-glucosamine	.93	+	+	−
DNP glucosamine diethyl mercaptal	.89	−	−	−
tetracetyl DNP glucosamine diethyl mercaptal	.96	−	−	−
DNP glucosaminic acid	.60	−	−	−
DNP chondrosamine	.61	+	+	−
1:3:4:6-tetracetyl DNP chondrosamine	.92	+	+	−
DNP glycine	.42	−	−	−
DNP tyrosine	.77	−	−	−
DNP histidine	(i) .64 (ii) .85	−	−	−
DNP arginine	.65	−	−	−
DNP valine	.72	−	−	−
DNP aspartic acid	.28	−	−	−
DNP alanine	.58	−	−	−
DNP tryptophane	0.69	−	−	−

readily separated using Whatman No. 1 filter paper and neutral N-butanol (40%)—ethanol (10%)—water (50%) as the eluting agent. The yellow band could be observed directly, and on spraying the chromatogram with aniline hydrogen phthalate the other sugars were revealed and the identity of the colored band confirmed as an amino sugar derivative by its specific color change from yellow to brown.

A typical mixture separated has the following composition: glucose (0.31 mg), rhamnose (1.44 mg), arabinose (0.26 mg), 2:3:4:6 tetramethyl glucose (0.23 mg), DNP tetracetylglucosamine (0.35 mg).

The reaction with 2:4-dinitrofluorobenzene could be carried in the presence of neutral sugars. Thus a mixture containing, for example, glucosamine (2 mg), arabinose (4 mg), glucose (10 mg), and glucuronic acid (5 mg) in 1.0 ml 1% sodium bicarbonate and 1.0 ml ethanol reacted at room temperature when shaken with 2:4-dinitrofluorobenzene (0.05 ml, i.e., excess). Two drops of the resulting reaction mixture were applied to the chromatogram. The DNP amino sugar was clearly separated, the unchanged fluoro reagent moved with the solvent front, and the remaining sugars were observed by treatment with aniline hydrogen phthalate.

It was also found possible to separate DNP glucosamine from DNP chondrosamine and free amino acids, the latter being observed by their coloration with ninhydrin.

The problem of the separation of DNP amino sugars and DNP amino acids (*6*) has been investigated. This involved the determination of the R_F values of numerous DNP amino acids as summarized in Table 1. It was found that a distinct separation could be achieved between many DNP amino acids, DNP amino sugars, and free amino acids. DNP amino acids were without effect on silver nitrate solution, aniline hydrogen phthalate, or ninhydrin.

The results are being applied to the investigation of the hydrolyzates from various mucopolysaccharides.

References

1. PARTRIDGE, S. M. *Nature,* **158**, 270 (1946); *Biochem. J.,* **42**, 238 (1948).
2. KENT, P. W. *Proc. 4th Intern. Cong. Microbiol., Copenhagen,* 157 (1947).
3. PASCU, E., MORA, T. P., and KENT, P. W. *Science,* **110**, 446 (1949).
4. PARTRIDGE, S. M. *Nature,* **161**, 443 (1949).
5. KENT, P. W. *Research, London,* **3**, 427 (1950).
6. SANGER, F. *Biochem. J.,* **39**, 507 (1945); *ibid.,* **45**, 563 (1949).

Proteins of Liver and Hepatoma Mitochondria

George H. Hogeboom and Walter C. Schneider

National Cancer Institute,
National Institutes of Health, Bethesda, Maryland

The cytoplasmic inclusions known under the term mitochondria or chondriosomes are among the most prominent and widely distributed structures of living cells (*1*). As a result of cytological studies carried out in the past 50 years, a number of theories have arisen concerning the physiological significance of mitochondria, including the still-tenable and intriguing view that they are self-duplicating and autonomous cell organelles (*2*). Until recently, however, most ideas pertaining to their function have been based largely on conjecture.

With the advent of improvements in the technique of cell fractionation by means of differential centrifugation, permitting the isolation of intracellular components in cytologically defined states (*3*), the importance of the role played by the mitochondrion in cellular metabolism has become increasingly apparent.