As far as the authors are aware, data on the concentration of alkali salts in ground level air are available from 16 different sites. The 15 investigators engaged in these studies have made a total of some 500 individual determinations. The data obtained in these studies are presented in Tables 1 and 2, which refer to coastal and inland sites, respectively. For convenience in the application of these results to background effects in flame spectroscopy (*4*), the data have been converted to common units of sodium atoms/cm^3 (Na/cm^3) under the assumption that $[Na^+]/[Cl^-] = 1$. The overall average of Na/cm^3 found for the various inland sites, is 1.5×10^{11} sodium atoms/cm^3.

The locations of the sites mentioned in Tables 1 and 2 are shown in the accompanying map (Fig. 1). It may be noted that these sites form a representative sample of test locations in the northern temperate zone.

References

1. BOSSOLASCO, M. *Z. Meteorol.*, **51**, 84 (1934).
2. GODARD, H. P., and SEYER, W. F. *Trans. Roy. Soc. Can., III*, **30**, Sec. 3, 85 (1936).
3. WOODCOCK, A. H. *Am. Soc. Testing Materials, Proc.*, **50**, 1151 (1950).
4. SHULER, K. E., and WEBER, J. *J. Phys. Chem.* To be published.
5. *The Smog Problem in Los Angeles County*, Stanford Research Inst., Third Interim Rept., 1950.
6. LANDSBERG, H. In: *Gerlands Beiträge zur Geophysik, Dritter Supplementband. Ergebnisse der Kosmischen Physik III. Physik der Atmosphäre.* V. Conrad, Ed. Leipzig: Akademische Verlagsgesellschaft, 1950.
7. NEUBERGER, H. *Mech. Eng.*, **70**, 221 (1948).
8. ———. *Penn. State Coll. Bull. Mineral Ind. Expt. Sta. Bull.*, **18**, (5), 1 (1949).
9. MATUI, H. *Meteorol. Soc. Japan, J., II*, **17**, 367. Suppl. (in English), 34 (1939).
10. ———. *Ibid.*, **19**, 72. Suppl. (in English), 7 (1941).
11. ALIVERTI, G. *Ricerca sci.*, **12**, 1251 (1941).
12. ———. *Ibid.*, **14**, 268 (1943).
13. BURKSER, E. S., and GERNET, E. V. *J. Geol. Ukrain. Acad. Sci., Inst. Geol.*, **6**, (3), 5 (in English, 21), (1940).
14. ———. *Chem. Abstr.*, **35**, 353 (1941).
15. JACOBS, W. C. *Monthly Weather Rev.*, 147 (1937).
16. ———. *Bull. Am. Meteorol. Soc.*, **17**, 301 (1936).
17. ———. *Z. Meteorol.*, **54**, 71 (1937).
18. ———. *Bull. Am. Meteorol. Soc.*, **20**, 38 (1939).
19. LOEWY, A., MÜLLER, F., and CRONHEIM, W. C. *Therap. Monatsh.*, **26**, 124 (1912).
20. TAKAYANAGI, G. *J. Chem. Soc. Japan*, **62**, 249 (1941).
21. ———. *Chem. Abstr.*, **35**, 4645 (1941).
22. OOSTING, H. J., and BILLINGS, W. D. *Ecology*, **23**, 131 (1942).
23. BOYCE, S. G. *Science*, **113**, 620 (1951).
24. ZANON, F. S. *Riv. ecol.*, **1**, (1–2), 66 (1949).
25. GAUTIER, A. *Bull. soc. chim. (Paris)*, **21**, 391 (1899).
26. WOODCOCK, A. H., and GIFFORD, M. M. *J. Marine Research Sears Foundation*, **8**, 177 (1949).
27. WOODCOCK, A. H. *J. Meteorol.*, **7**, 161 (1950).
28. ———. *Ibid.*, **7**, 397 (1950).
29. ———. *Ibid.*, **9**, 200 (1952).
30. SUGAWARA, K., OANA, S., and KOYAMA, T. *Bull. Chem. Soc. Japan*, **22**, 47 (1949).
31. SUGAWARA, K. *J. Japan. Chemistry*, **2**, 341 (1948).
32. ———. *Chem. Abstr.*, **45**, 4499 (1951).
33. MIJAKE, Y. *Geophys. Mag. (Tokyo)*, **16**, 64 (1948).
34. SOUDAIN, G. *J. sci. météorol.*, **3**, (12), 137 (1951).
35. VONNEGUT, B., and NEUBAUER, R. L. *Bull. Am. Meteorol. Soc.*, **34**, 163 (1953).

Some Carbohydrate Components of Reticular Fibers[1]

R. E. Glegg, D. Eidinger, and C. P. Leblond

Department of Anatomy, McGill University, Montreal, Canada

OVER HALF A CENTURY AGO, Mall (*1*) showed that connective tissue contains translucent netlike "reticular fibers," as distinct from the white bundles of collagenous fibers; and Siegfried (*2*) isolated from reticular fibers a material which he called "reticulin." A few years later, histologists showed that in sections of various tissues treated by the silver method of Bielschowsky, reticular fibers stain black,[2] while collagenous fibers stain light brown (*3–7*). This has remained the only universally accepted criterion for the distinction between these fibers. There were either no or negligible differences when the two types of fibers were compared by the usual staining techniques (*8*), by chemical methods of analysis (*9*), or by the techniques of electron microscopy (*10–12*) and x-ray diffraction (*13–15*).

There is a widespread opinion that even the result obtained with the silver method does not reflect true chemical differences between the two types of fibers. Instead, it is attributed to a physical effect related to fiber diameter, with the smaller reticular fibers presenting a greater surface for the precipitation of silver than the larger collagenous fibers, and thus appearing black instead of light brown (*8, 16*).

However, the fact that the periodic acid-Schiff technique stains reticular fibers intensely (*17, 18*) and collagenous fibers only faintly (*18*) suggested the existence of some chemical differences. The evidence accumulated by the work of several investigators (*19–21*) indicates that in routine histological sections, the periodic acid-Schiff technique detects 1,2-glycol and α-amino alcohol groups (which are oxidized by periodic acid to yield aldehyde groups which

[1] This work was supported by a grant from the National Cancer Institute of Canada. The cattle tissues were obtained with the kind cooperation of H. Nadeau and G. McDavid of Canada Packers, Ltd. The Permutit Q was a gift from the Permutit Company.

[2] For convenience, the term "reticular fibers" is used in the present work to include fibers as well as the membranous structures stained by the silver method: basement membrane, membrana propria, reticulum. Rühle (*34*) and many others feel that these membranes are composed of reticular fibers.

in turn react with the Schiff reagent); and furthermore, that these reactive groups occur mainly within tissue carbohydrates. Thus, the two types of fibers would differ qualitatively or quantitatively in their carbohydrate components.

In the present work, the presence of carbohydrate components in reticular and collagenous fibers was investigated by paper chromatography. The results provided a basis for a chemical distinction between the two types of fibers.

On examination of a series of cattle tissues stained by the silver method (*7*), it was found that lymph node, lung, testis, and adipose tissue (perirenal fat) contained an abundance of reticular and some collagenous fibers. These tissues were, therefore, selected for carbohydrate analysis. Achilles tendon was used as a source of collagenous fibers since it contained almost exclusively this type of fiber, although small amounts of reticular fibers are also present (*22*).

The techniques used were not devised to separate "reticulin" and "collagen" chemically, but rather to obtain whatever reticular and collagenous fibers there were in the organ as cell-free as possible. Treatment with acids, alkalis, or enzymes, was avoided since these agents might cause destruction of some of the components.

The organs and tissues were stripped of their capsules and adhering material. Large blood vessels were excised. The lymph nodes, lungs, testes, and tendons were cut into small pieces and repeatedly pressed in a Latapie plastic squeezer and washed with large volumes of cold running water to remove cells and soluble materials. They were then washed with distilled water, dehydrated with alcohol, fat extracted with boiling acetone, washed in ether, and dried. The adipose tissue was subjected only to fat extraction, ether washing, and drying. The residues obtained after extraction of lymph node, lung, testis, and adipose tissue mainly consisted of reticular fibers and membranes, with a fair amount of collagenous fibers, and are referred to as reticular materials. In contrast, the residue from the extraction of tendon consisted essentially of collagenous fibers and is referred to as collagenous material.

The hydrolysis of these materials was catalyzed by the hydrogen form of a polystyrene sulfonic acid resin, Permutit Q (*23*). The resin was pretreated with 4.4*N* hydrochloric acid (900 ml of acid per liter of resin), washed with distilled water until the washings were neutral and free from chloride ions, and air-dried. Two hundred milligrams of each of the materials prepared, 2.4 g of resin, and 5 ml of water were heated in sealed glass tubes in an oven at 100° for 48 hours. The liquid was decanted, the resin was washed twice in the tube with 2-ml portions of water, the solutions were combined and filtered. The filtrate was evaporated to dryness *in vacuo* at 40°C, and the residue was dissolved in 0.1 ml water. The solution was analyzed by unidimensional ascending paper chromatography, using rectangular sheets of Whatman No. 1 ashless paper, 27 cm wide by 38 cm high. Three λ of each of the solutions were placed at individual points of origin located on a line drawn at 4 cm from one of the narrow edges of the paper. For comparison, 3 λ of a solution which contained 1% of each one of the following sugars, galactose, glucose, mannose, fucose, and ribose were also placed on the line as a marker. The paper was fashioned into a cylinder by stapling the long edges together, developed three times (*24*) in a butanol: pyridine: water solvent (*25*), and sprayed with the aniline hydrogen oxalate reagent (*26*). Simple sugars, and hexuronic acids, but not hexosamines, can be recovered and detected by this method of hydrolysis and chromatography.

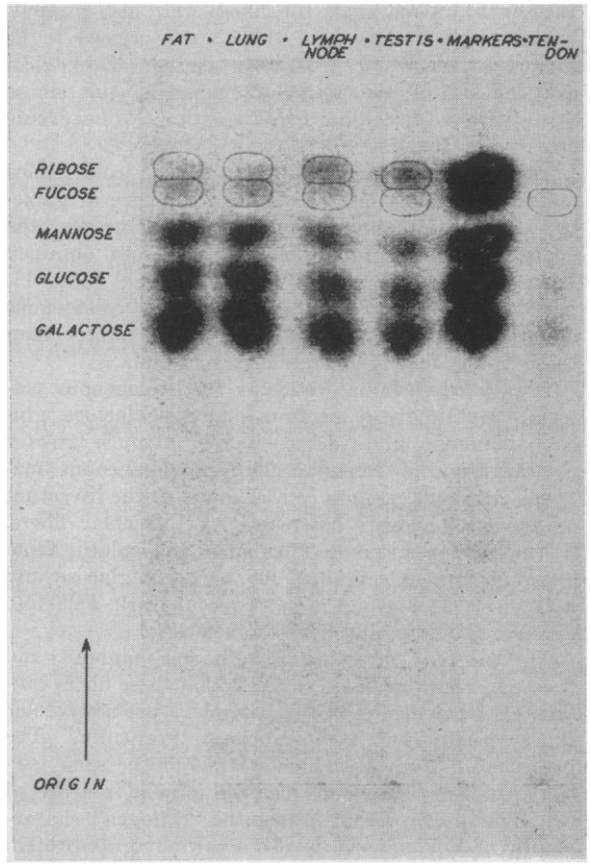

FIG. 1. Paper chromatogram. The markers indicate the location of the galactose, glucose, and mannose spots, while the fucose and ribose spots, which on the paper could be easily distinguished by their greenish and reddish color, respectively, appear fused on the photograph. The positions of the weak fucose and ribose spots in hydrolyzed materials are outlined.

On the left of the markers, hydrolyzates of various organ preparations rich in reticular fibers show the presence of large amounts of galactose, glucose, and mannose and small amounts of fucose and ribose. On the right of the markers, a hydrolyzate of a tendon preparation rich in collagenous fibers shows the presence of traces of galactose, glucose, mannose, and fucose.

The reticular materials (Fig. 1) gave rise to intense reddish-brown spots for galactose, glucose, and mannose and a less intense greenish-brown spot for

fucose.[3] Faint red spots for ribose were also present, but since the intensity of these spots seemed to be related to the number of cells seen on histological examination of the reticular materials, the ribose was attributed to cellular contamination. No hexuronic acids were found.

The chromatogram from 200 mg of collagenous material (tendon) also showed the presence of galactose, glucose, mannose, and fucose. However, the spots were much less intense than those obtained from the same weight of reticular material (Fig. 1). Even when five times this amount of collagenous material was used, the spots were still fainter than those from 200 mg of reticular material.

It is concluded that reticular and collagenous materials contain the same four sugars—galactose, glucose, mannose, and fucose—but in a much greater concentration in reticular than in collagenous material. Although no data can be found in the literature on the carbohydrate components of reticular fibers, it has been reported that collagen and gelatin from different sources contained 0.5 per cent glucose and galactose (*27, 28*) and in more recent work, galactose glucose, mannose, and hexosamine (*29*).

The presence of galactose, glucose, mannose, and fucose in reticular fibers suggests that these fibers contain a carbohydrate-protein complex, probably their characteristic chemical component, "reticulin." The presence of a much smaller amount of the same carbohydrates in collagenous material may be interpreted in either of two ways. Either the "collagen" characteristic of this material is also a carbohydrate-protein complex with a much lower content of carbohydrates than "reticulin" or it is a protein contaminated with reticulin due to the presence of some reticular fibers within all types of connective tissue (*30, 31*), even tendon (*22*).

Accordingly, the pronounced staining of reticular fibers and membranes by the periodic acid-Schiff technique is explained by their high content of carbohydrate, while the slight staining of collagenous fibers by this technique is due to the small amount of carbohydrate which is either incorporated in, or a contaminant of, collagen. The same explanation may account for the results of the silver stain since at least in its later modifications, it includes—like the periodic acid-Schiff technique—the successive action of an oxidizing agent (potassium permanganate [*6, 7*] or periodic acid [*32, 33*]) and an aldehyde reagent (ammoniacal silver nitrate).[4]

In conclusion, carbohydrates are present in large amounts in "reticulin" and in small amounts (possibly as a contaminant) in "collagen." These findings satisfactorily explain some of the staining properties of reticular and collagenous fibers.

[3] Control experiments revealed that fucose is partly destroyed by heating with the resin; therefore, a larger amount may be present than appears from the chromatograms.

[4] It is probable that the two types of fibers have a similar protein moiety (*9–15*). This might explain why histological techniques other than the silver method and periodic acid-Schiff technique stain them in the same manner (*8*).

References

1. MALL, F. *Abhandl. math. phys. Klasse sächs. Ges. Wiss.*, **17**, 295 (1891).
2. SIEGFRIED, M. *Habilitationsschrift.* Leipzig: F. A. Brockhaus (1892).
3. MARESCH, R. *Zentr. path. Anat.*, **16**, 641 (1905).
4. RÖSSLE, R., and YOSHIDA, T. *Beitr. pathol. Anat. u. allgem. Pathol.*, **45**, 110 (1909).
5. RUSSAKOFF, A. *Ibid.*, 476 (1909).
6. FOOT, N. C. *Am. J. Pathol.*, **4**, 525 (1928).
7. GOMORI, G. *Ibid.*, **13**, 993 (1937).
8. MALLORY, F. B., and PARKER, F. *Ibid.*, **3**, 515 (1927).
9. BOWES, J. H., and KENTEN, R. H. *Biochem. J. (London)*, **45**, 281 (1949).
10. GROSS, J. *Ann. N. Y. Acad. Sci.*, **52**, 964 (1950).
11. LITTLE, K., and KRAMER, H. *Nature*, **170**, 499 (1952).
12. TOMLIN, S. G. *Ibid.*, **171**, 302 (1953).
13. BEAR, R. S. *Advances in Protein Chem.*, **7**, 69 (1952).
14. LLOYD, D. J. *Progress in Leather Science*, 1920–1945. Ch. 3. London: British Manufacturer's Research Assoc.
15. GROSS, J. *J. Gerontol.*, **5**, 343 (1950).
16. WOLBACK, S. B. *Am. J. Pathol.*, **9**, 689 (1933).
17. LILLIE, R. D. *J. Lab. Clin. Med.*, **32**, 910 (1947).
18. LEBLOND, C. P. *Am. J. Anat.*, **86**, 1 (1950).
19. HOTCHKISS, R. D. *Arch. Biochem.*, **16**, 131 (1948).
20. GLEGG, R. E., CLERMONT, Y., and LEBLOND, C. P. *Stain Technol.*, **27**, 277 (1952).
21. MCMANUS, J. F. A., and CASON, J. E. *J. Exptl. Med.*, **91**, 651 (1950).
22. BATE-SMITH, E. C. *J. Intern. Soc. Leather Trades' Chemists*, **31**, 161 (1947).
23. GLEGG, R. E., and EIDINGER, D. Unpublished.
24. JEANES, A., WISE, C. S., and DIMLER, R. J. *Anal. Chem.*, **23**, 415 (1951).
25. CHARGAFF, E., LEVINE, G., and GREEN, C. *J. Biol. Chem.*, **175**, 67 (1948).
26. HORROCKS, R. H., and MANNING, G. B. *Lancet*, **256**, 1042 (1949).
27. GRASSMAN, W., and SCHLEICH, H. *Biochem. Z.*, **277**, 320 (1935).
28. BEEK, J. *J. Am. Leather Chemists' Assoc.*, **36**, 696 (1941).
29. GROSS, J., HIGHBERGER, J. H., and SCHMITT, F. O. *Proc. Soc. Exptl. Biol. Med.*, **80**, 462 (1952).
30. KAYE, M. *J. Intern. Soc. Leather Trades' Chemists*, **20**, 223 (1936).
31. RODDY, W. T., and O'FLAHERTY, F. *J. Am. Leather Chemists' Assoc.*, **34**, 671 (1939).
32. GRIDLEY, M. F. *Am. J. Clin. Pathol.*, **21**, 897 (1951).
33. LHOTKA, J. F. *Stain Technol.*, **28**, 129 (1952).
34. RÜHLE, G. *Arch. Entwicklungsgmesch.*, **170**, 153 (1897).

Fact without theory is chaos. Theory without fact is fantasy.

C. O. WHITMAN