The Syntheses of 1-[^{11}C]-D-Glucose and Related Compounds for the Measurement of Brain Glucose Metabolism

Chyng-Yann Shiue and Alfred P. Wolf

Department of Chemistry, Brookhaven National Laboratory

Upton, New York 11973 USA

SUMMARY

A new synthesis of 1-[^{11}C]-D-glucose (3) and related compounds from H^{11}CN is described. Reaction of D-arabinose (1) with Na^{11}CN at pH 8 gives 1-[^{11}C]-aldononitriles (2). Reduction of 2 with Raney alloy in 30% formic acid gives 1-[^{11}C]-D-glucose (3) and 1-[^{11}C]-D-mannose (4) in radiochemical yields of ~ 40-50% (EOB) in a synthesis time of 50 min from EOB. The yield and ratio of 3 and 4 is pH dependent. Compounds 3 and 4 are separated by HPLC. The radiochemical yield of 3 is ~ 15% (EOB) and the total synthesis time (including HPLC purification) is 70 min from EOB. The same method has also been applied to synthesize 1-[^{11}C]-D-galactose (6) with radiochemical yields of ~ 30% (EOB) in a synthesis time of 70 min from EOB. The advantages and disadvantages of this synthetic method are discussed.

Key Words: 1-[^{11}C]-D-Glucose; 1-[^{11}C]-D-Mannose, 1-[^{11}C]-D-Galactose; Kiliani-Fischer cyanohydrin synthesis; molybdate ion catalysis.

INTRODUCTION

The rapid development in the technology of positron emission tomography (PET) has made it possible to study the dynamic properties of radiopharmaceuticals in vivo non-invasively and quantitatively. For example, several glucose analogs labeled with positron emitting nuclei have been used to study regional brain glucose metabolism non-invasively in humans (1-3). Among these, 2-deoxy-2-[^{18}F]fluoro-D-glucose (4-13) and 1-[^{11}C]-2-deoxy-D-glucose (14-17) have been used as tracers for quantitatively mapping the first step of glycolysis in the brain and the heart. The compound 3-deoxy-3-[^{18}F]fluoro-D-glucose (18,19) has also been suggested for utilization. More recently, 3-[^{11}C]methyl-D-glucose has been prepared and evaluated as a tracer for glucose transport (20,21). Nevertheless, these compounds are not natural substances, rather, they are the analogs of glucose. There are several advantages and disadvantages associated

0362-4803/85/020171-12$01.20

Received August 16, 1984

with these analogs (22). The most significant disadvantage associated with analogs is their lack of biochemical identity. Thus it is necessary to correct for differences in transport properties and enzyme affinities which vary among species. Since such corrections may present more difficulties when the tissue of interest is diseased, a positron-emitter labeled glucose itself, rather than an analog is an attractive tracer for the measurement of regional glucose metabolism in humans even though the complexity of its metabolism makes interpretation of results more difficult (23-25).

Carbon-11 labeled glucose has been synthesized by a biosynthetic method (26-28). However, there are at least two problems associated with this method: (a) the glucose is randomly labeled but may not be uniformly labeled, (b) the tedium and uncertainties introduced by the uniformity of the plant materials used for the biosynthesis resulting in the irreproducibility of the synthesis. The use of algae to prepare ^{11}C-galactose has been reported (29). Recently, ^{11}C-labeled glucose has also been synthesized from $H^{11}CO_3^-$ utilizing algae as the biosynthetic medium (30) but seems to afford no special advantages. However, a chemical synthesis which can overcome these problems may be advantageous. For these reasons, we have developed a synthesis of 1-[^{11}C]-D-glucose (3) and related compounds from [^{11}C]NaCN by modifying the classical Kiliani-Fischer cyanohydrin synthesis. Preliminary reports of the application of this synthesis to the preparation of 1-[^{11}C]-D-glucose (3) have appeared (31,32).

MATERIALS AND METHODS

D-Arabinose, D-galactose, D-glucose, D-lyxose, D-mannose and D-talose were purchased from Sigma Chemical Company, and were used without further purification. GLC analyses were carried out with a Hewlett-Packard 5830A gas chromatography equipped with a thermal conductivity detector. A column [1.8 m x 3 mm] containing SE-30 (10%) on Chromosorb 80/100 mesh was employed, isothermal at 190° and a flow of 50 ml/min. HPLC analyses were carried out either with a Waters Associates model 6000 liquid chromatography equipped with a refractive index detector or with a Perkin-Elmer Series 3B liquid chromatographs

equipped with a Berthold LB503 radioactivity monitor. A Bio-Rad HPLC carbohydrate analysis column (Aminex carbohydrate HPX-87, 300 mm x 7.8 mm) was employed and eluted with H_2O (85°). NMR spectra were recorded with a JEOL MH-100 spectrometer in D_2O with DSS as an internal standard.

Synthesis of glucose (3). A solution of 646.82 mg (12.3 mmol) of NaCN in 5 ml of H_2O was adjusted to pH 8.09 with 3 M HOAc (4.2 ml) and then 652.00 mg (4.34 mmol) of D-arabinose (1) in 5 ml of H_2O was added. The solution was stirred at room temperature for 35 min and then a suspension of Raney alloy (1.03 g) in 30% formic acid (45 ml) was added. The mixture was stirred for 1 hr at 100-110°, cooled to room temperature, passed through celite, and the green solution was evaporated *in vacuo* to dryness. The residue was dissolved in water, passed through a column (2 x 17 cm) of Dowex AG50W-X8 (H^+) ion-exchange resin and eluted with water (80 ml). The eluate was evaporated to dryness to give 776.2 mg (99.3%) of a mixture. HPLC (0.2 ml/min) analysis of the mixture showed peaks at R_T 16.25, 30.5, 33.5, 37 and 39.25 min. The first four peaks correspond to aldonic acids, glucose (3), mannose (4) and unreacted arabinose (1) respectively. The fifth peak (R_T = 39.25 min) was unidentified. Compounds 3 and 4 were isolated by HPLC (0.2 ml/min) to give 98 mg (12.5%) of 3 and 164 mg (21%) of 4. The identities of 3 and 4 were confirmed by GLC (silyl derivative) R_T = 16 min (α) and 23.31 min (β) for 3; and 11.52 min (α) and 16.79 min (β) for 4 and by HPLC and NMR.

Synthesis of galactose (6). A solution of 691.44 mg (14.11 mmol) of NaCN in 5 ml of H_2O was adjusted to pH 7.99 with 3 M HOAc (4.3 ml) and then 750.67 mg (5.00 mmol) of D-lyxose (5) in 5 ml of H_2O were added. The solution was stirred at room temperature for 50 min and then added into the suspension of Raney alloy (1.13 g) in 30% formic acid (45 ml). The mixture was stirred for 1 hr at 120°C. Work-up was the same as that for glucose to give 861.2 mg (95.6%) of a mixture. HPLC (0.2 ml/min) analysis of the mixture showed peaks at R_T 17.6, 33.75, 37.5 and 41 min. These peaks correspond to aldonic acids, galactose (6), lyxose (5) and talose (7). Compound 6 (350 mg, 38.9%) was isolated by HPLC (0.2 ml/min) and

was identified by GLC (silyl derivative) R_T = 14.63 min (α) and 18.16 min (β), HPLC and NMR. Talose (7) was formed in insignificant amounts. The ratio of 6 to 7 was ~ 17:1.

Interconversion of mannose and glucose: The method of Bilik (33) was adapted for this reaction. In a typical experiment, a solution of 102 mg of D-mannose and 3.92 mg of molybdic acid in 1 ml of H_2O was kept at 85°C. At different time intervals, an aliquot was taken and injected into HPLC. The percent of conversion was determined from the area ratio of glucose and mannose and the results were listed in Table 1. The conversion of glucose to mannose was also determined and the results were also listed in Table 1.

Table 1 Interconversion of Mannose and Glucose Catalyzed by Molybdic Acid at 85°C

Time (Min)	% Conversion	
	(Mannose → Glucose)	(Glucose → Mannose)
5	3.2	8.5
10	4.5	13.0
20	6.1	14.5
30	9.3	18.7
40	11.4	21.2
60	16.8	22.8
120	24.3	26.7
180	41.9	27.8
240	54.5	28.0
300	57.4	----
360	63.7	----
420	68.1	----
480	70.2	----

1-[^{11}C]-D-glucose (3) and 1-[^{11}C]-D-mannose (4). No-carrier-added (NCA) H^{11}CN was tranferred into 300 µl (0.34 mmol) of NaCN solution (pre-adjusted to pH 8.0 with 3 M HOAc) and then 300 µl (0.2 mmol) of D-arabinose solution was added. The solution was stirred at room temperature for 10 min, a suspension of 0.1 g of Raney alloy in 3 ml of 30% HCO_2H was then added. The mixture was stirred at 110°C for 10 min, cooled to room temperature and passed through AG50W-X8 (H^+) and

AG1-X8 (HCO_3^-) columns. The columns were eluted with an additional 5 ml of water. The combined effluent was concentrated and applied to HPLC (0.6 ml/min). The peaks corresponding to 1-[^{11}C]-D-glucose (3) (R_T = 10 min) and 1-[^{11}C]-D-mannose (4) (R_T = 11.5 min) were collected. The final products 3 and 4 also contain D-arabinose (R_T = 12.5 min) as a chemical impurity as detected by GLC.

In a typical experiment, from 22.14 mCi of H^{11}CN (at EOB), 2.45 mCi (at EOS) of 1-[^{11}C]-D-glucose (3) and 1-[^{11}C]-D-mannose (4) were isolated in a synthesis time of 46 min from EOB. The mixture was then separated by HPLC to give 0.3 mCi of 3 and 0.7 mCi of 4. The total synthesis time (including HPLC purification) of 3 was ~ 70 min from EOB. Thus, the radiochemical yield of 3 was 14.5% EOB.

1-[^{11}C]-D-galactose (6). No-carrier-added (NCA) H^{11}CN (16.6 mCi at EOB) was transferred into 300 µl (0.34 mmol) of NaCN solution (pre-adjusted to pH 8.0 with 3 M HOAc), and then 200 µl (0.13 mmol) of D-lyxose solution was added. The solution was stirred at room temperature for 10 min, a suspension of 0.1 g of Raney alloy in 3 ml of 30% HCO_2H was then added. The mixture was stirred at 110°C for 10 min, cooled to room temperature and passed through AG50W-X8 (H^+) and AG1-X8 (HCO_3^-) columns. The columns were eluted with an additional 5 ml of water. The combined effluent was concentrated and applied to HPLC (0.6 ml/min). The peak corresponding to 1-[^{11}C]-D-galactose (6) (R_T = 11.5 min) was collected to give 0.4 mCi of 6 in a synthesis time of 70 min from EOB. The radiochemical yield of 6 was 26% EOB. The final product 6 also contains D-lyxose (R_T = 13.5 min) as a chemical impurity as detected by GLC.

RESULTS AND DISCUSSION

Glucose and galactose have been synthesized by numerous methods. The first chemical synthesis was reported by Emil Fischer during the latter part of the 19th century (34,35) and has frequently been adapted in the synthesis of aldoses. The synthesis involves the addition of sodium cyanide with a parent aldose to produce, after alkaline hydrolysis of the nitriles, two epimeric aldonic acid salts which are separated and converted into lactones. The lactones, or their acylated derivatives, are then reduced to give aldoses. Although this method has been adapted for the synthesis of ^{14}C-labeled glucose

labeled at various positions (36), it is not suitable for the synthesis of glucose labeled with ^{11}C because of the 20.4 min half-life time constraints. Recently, Barker and his co-workers have developed an elegant method for the synthesis of ^{13}C-labeled carbohydrates (37). The synthesis involves the reaction of arabinose with [^{13}C]NaCN at controlled pH to give aldononitriles in high yield at a short period of time. This method has been adapted for the syntheses of 4-deoxy-4-fluoro-D-[1-^{14}C]glucose (8) and 4-deoxy-4-fluoro-D-[1-^{14}C]mannose (9) (38). Unfortunately, the reduction of aldononitriles to glucose and mannose took more than 2 hrs. Nevertheless, we have adapted this method to prepare 1-[^{11}C]-D-glucose (3), 1-[^{11}C]-D-mannose (4) and 1-[^{11}C]-D-galactose (6) with some minor modifications.

Reaction of D-arabinose (1) with Na^{11}CN (39) at pH 8 at room temperature for 10 min gave aldononitriles (2) in good yield. Reduction of 2 to 1-[^{11}C]-D-glucose (3) and 1-[^{11}C]-D-mannose (4) was accomplished by a modification of the procedure of Van Es and Staskun (15,40), which involved heating 2 in aqueous formic acid in the presence of Raney alloy (Fig. 1), a reduction method which is now routinely used in the rapid synthesis of 1-[^{11}C]-2-deoxy-D-glucose (15). Filtration of this reaction mixture through a cation-exchange resin (hydrogen form) removed dissolved metal ions and the C-11 amines that were formed as by-products during the reduction. Passage of the effluent of the cation-exchange resin through an anion-exchange resin (bicarbonate form) neutralized the acidic

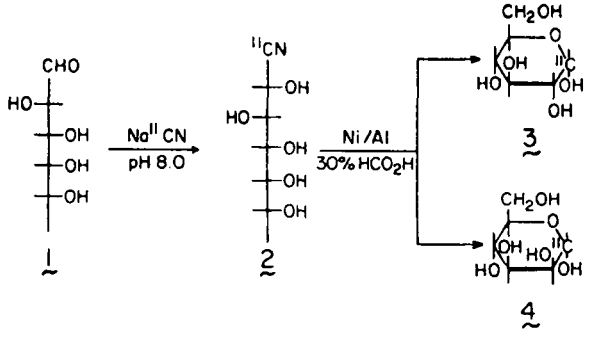

FIGURE 1
SYNTHESES OF 1-[^{11}C]-D-GLUCOSE AND 1-[^{11}C]-D-MANNOSE

solution and removed aldonic acids that were formed as by-products during reduction/hydrolysis. The recovery of C-11 activity from the system was taken as the sum of the activities from the cation-exchange resin, the anion-exchange resin, the reaction-vessel walls, the soda lime and the effluent from the ion-exchange columns, and is shown in Table 2. The overall radiochemical yields of 3 and 4 were 40-50% (EOB) in a synthesis time of 50 min from EOB. Final purification was achieved by HPLC. The radiochemical yield of 3 was ~ 15% (EOB) and the total synthesis time (including HPLC purification) was ~ 70 min from EOB. These results were comparable with the results obtained from the reaction of K^{14}CN with 3-deoxy-3-fluoro-D-arabinose which gave compounds 8 and 9 with radiochemical yields of 11% and 35%, respectively. 1-[^{11}C]-D-Galactose (6) has been synthesized by the same method with a radiochemical yield of ~ 30% (EOB) in a synthesis time of 70 min from EOB.

Table 2 Distribution of Carbon-11 Activities in the Synthesis of 1-[^{11}C]-D-Glucose and Related Compounds*

	Distribution of C-11 (%)
Cation-exchange resin	27.3 ± 3.7
Anion-exchange resin	5.4 ± 2.3
Reaction-vessel walls	6.6 ± 3.2
Soda lime	5.2 ± 3.3
Products (glucose + mannose)	55.6 ± 4.6

* Based on total H^{11}CN activities recovered from H_2/N_2 target (39a,b). The yield data represent a mean ± standard deviation of 5 runs.

The 1-[^{11}C]-D-glucose and 1-[^{11}C]-D-mannose synthesized by this method contained D-arabinose as a chemical impurity. Since D-arabinose is non-radioactive and nontoxic (LD_{50} in dogs 5 g/kg), it would neither interfere with PET studies nor have pharmacological effects on human subjects.

The yield and ratio of glucose to mannose is pH dependent. At lower pH (pH 8-9), glucose and mannose are the major products, and the ratio of glucose to mannose is ~ 1:2. At higher pH (pH > 11), aldonic acids become the major products at the expense of glucose and mannose, and the ratio of glucose to mannose is ~ 1:1. The yield of glucose and mannose, also depends on the ratio of

cyanide to arabinose as illustrated in Table 3. When the molar ratio of cyanide to arabinose is ~ 1:1, 50% of the arabinose is recovered. However, as the molar ratio of cyanide to arabinose increases, the yields of glucose and mannose increase. Therefore, in the syntheses of unlabeled glucose and mannose, an excess of cyanide should be used. However, in the synthesis of ^{11}C-labeled compounds, an excess of pentose should be employed to improve ^{11}C-cyanide incorporation.

Table 3. Distribution of products in the reaction of D-arabinose with sodium cyanide at pH 8.0 as a function of [NaCN]/[D-arabinose]

[NaCN]/[D-arabinose]	1	2	3	4
% unreacted D-arabinose (1)	46.8	17.2	9.1	10.5
% glucose (3)	18.5	28.3	30.8	32.3
% mannose (4)	34.6	54.5	60.2	57.3

The specific activities of 1-[^{11}C]-D-glucose (3) and 1-[^{11}C]-D-mannose (4) are variable, depending on the length of the cyclotron bombardment. Although the method reported here can be used to produce compounds 3 and 4 in very high specific activities, glucose is ubiquitous in mammals and dilution occurs immediately on injection, it is not necessary to prepare compound 3 with very high specific activity. Nevertheless, this new synthetic method has several advantages over the biosynthetic methods: (a) The method can be used to label glucose specifically at the one position. This is especially important, since earlier studies with glucose labeled with ^{14}C in specific positions indicated that these specific labeled glucoses can be used as probes for various pathologies (25,41-44). By labeling glucose with ^{11}C at various positions, it may be possible to distinguish individual pathways, both normal and pathological states. (b) Position specific labeling of glucose by synthetic methods also allows the preparation of very much larger quantities of the radioactive material without the tedium and uncertainties introduced by utilizing biosynthesis with plant or animal materials. (c) Certainty about the label position is assured by

the method of synthesis. The disadvantage of this method, as discussed by Rasmussen (38), is that a pair of epimers is produced with mannose as a major product, thus necessitating chromatographic separation and lowering the overall yield. Although it is possible to convert mannose into glucose by molybdate ions (33) (Table 1), this process is impractical for mannose labeled with ^{11}C because of the 20.4 min half-life time constraints. Work is in progress to label other positions in the glucose molecule with positron emitters.

ACKNOWLEDGEMENTS

This research was carried out at Brookhaven National Laboratory under contract with the U. S. Department of Energy (No. DE-AC02-76CH00016) and supported by its Office of Health and Environmental Research. The authors thank Dr. J. S. Fowler for her constructive comments.

REFERENCES

1. Reivich M., Kuhl D., Wolf A.P., Greenberg J., Phelps M., Ido T., Casella V., Fowler J., Hoffman E., Alavi A., Som P. and Sokoloff L. - Circ. Res. 44: 127 (1979).
2. Phelps M.E., Huang S.-C., Hoffman E.J., Selin C., Sokoloff L., and Kuhl D.E. - Ann. Neurol. 6: 371 (1979).
3. Reivich M., Alavi A., Wolf A., Greenberg H.H., Fowler J., Christman D., MacGregor R., Jones S.C., London J., Shiue C.-Y., and Yonekura Y. - J. Cereb. Blood Flow Metab. 2: 307 (1982).
4. Ido T., Wan C.-N., Casella V., Fowler J.S., Wolf A. P., Reivich M., and Kuhl D.E. - J. Label. Cmpds. Radiopharm. 14: 175 (1978).
5. Barrio J.R., MacDonald N.S., Robinson G.D., Najafi A., Cook J.S., and Kuhl D.E. - J. Nucl. Med. 22: 372 (1981).
6. Fowler J.S., MacGregor R.R., Wolf A.P., Farrell A. A., Karlstrom K.I., and Ruth T.J. - J. Nucl. Med. 22: 376 (1981).
7. Shaughnessy W.J., Gatley S.J., Hickwa R.D., Lieberman L.M. and Nickles R.J. - Int. J. Appl. Radiat. Isot. 32: 23 (1981).

8. Shiue C.-Y., Salvadori P.A., Wolf A.P., Fowler J.S., and MacGregor R.R. - J. Nucl. Med. 23: 899 (1982).

9. Shiue C.-Y., To K.-C., and Wolf A.P. - J. Label. Cmpds. Radiopharm. 20: 157 (1983).

10. Levy S., Elmaleh D.R. and Livni E. - J. Nucl. Med. 23: 918 (1982).

11. Diksic M. and Jolly D. - Int. J. Appl. Radiat. Isot. 34: 893 (1983).

12. Sood S., Firnau G., and Garnett E.S. - Int. J. Appl. Radiat. Isot. 34: 743 (1983).

13. Tewson T. J. - J. Nucl. Med. 24: 718 (1983).

14. Shiue C.-Y., MacGregor R.R., Lade R.E., Wan C.-N., and Wolf A.P. - J. Nucl. Med. 19: 676 (1978).

15. MacGregor R.R., Fowler J.S., Wolf A.P., Shiue C.-Y., Lade R.E., and Wan C.-N. - J. Nucl. Med. 22: 800 (1981).

16. Padgett H.C., Barrio J.R., MacDonald N.S., and Phelps M.E. - J. Nucl. Med. 23: 739 (1982).

17. Vora M.M., Boothe T.E., Finn R.D., Smith P.M., and Gilson A.J. - J. Label. Cmpds. Radiopharm. 20: 417 (1983).

18. Tewson T.J., Welch M.J. and Raichle M.E. - J. Nucl. Med. 19: 1339 (1978).

19. Goodman M.M., Elmaleh D.R., Kearfott K.J., Ackerman R.H., Hoop B., Brownell G.L., Alpert N.M., and Strauss H.W. - J. Nucl. Med. 22: 138 (1981).

20. Kloster G., Muller-Platz C., and Laufer P. - J. Label. Cmpds. Radiopharm. 18: 855 (1981).

21. Laufer P. and Kloster G. - Int. J. Appl. Radiat. Isot. 23: 775 (1982).

22. Raichle M.E. - Brain Res. Rev. 1: 47 (1979).

23. Hawkins R., Hass W.K., Ransohoff J. - Stroke 10: 690 (1979).

24. Raichle M.E., Larson K.B., Phelps M.E., Grubb R.L., Welch M.J., and Ter-Pogossian M.M. - Am. J. Physiol. 228: 1936 (1975).

25. Sacks W. - J. Appl. Physiol. 10(1): 37 (1957).

26. Lifton J.F. and Welch M.J. - Radiat. Res. 45: 35 (1971); cf. also Straatmann M.G. and Welch M.J. - Int. J. Appl. Radiat. Isot. 24: 234 (1973).

27. Wolf A.P. - Radioisotopy 12: 499 (1971).

28. Goulding R.W. and Palmer A.J. - Int. J. Appl. Radiat. Isot. 24: 7 (1973).

29. Palmer A.J. and Goulding R.W. - J. Label. Cmpds. Radiopharm. 10: 627 (1974).

30. (a) Ehrin E., Westman E., Nilsson S.O., and Nilsson J.L.G. - J. Label. Cmpds. Radiopharm. 17: 453 (1980). (b) Ehrin E., Stone-Elander S., Nilsson J.L.G., Bergström M., Blomqvist G., Brismar T., Eriksson L., Grenz T., Jansson P., Litton J.-E., Malmborg P., Ugglas M., and Widen L. - J. Nucl. Med. 24: 326 (1983).

31. Shiue C.-Y. and Wolf A.P. - J. Nucl. Med. 22: p58 (1981).

32. Shiue C.-Y. and Wolf A.P. - 182nd ACS National Meeting, New York, NY, August 23-28, 1981. Nucl. 52.

33. Bilik V., Petrus L., and Farkas V. - Chem. Zvesti. 29: 690 (1975).

34. Fischer E. - Chem. Ber. 22: 2204 (1889).

35. Kiliani H. - Chem. Ber. 18: 3066 (1885); cf. also Kiliani H. - Chem. Ber. 19: 3029 (1886).

36. Isbell H.S., Karabinos J.V., Frush H.L., Holt N.B., Schwebel A., and Galkowski T.T. - J. Res. Natl. Bur. Std. 48: 163 (1952); cf. also Isbell H.S., Frush H.L., Holt N.B. - J. Res. Natl. Bur Std. 53: 217 (1954).

37. Serianni A.S., Nunez H.A., Barker R. - Carbohydr. Res. 72: 71 (1979); cf. also Serianni A.S., Nunez H.S., Barker R. - J. Org. Chem. 45: 3329 (1980).

38. Rasmussen J.R., Tafuri S.R., and Smale S. T. - Carbohydr. Res. 116: 21 (1983).

39. (a) Christman D.R., Finn R.D., Karlstrom K.I., and Wolf A.P. - J. Nucl. Med. 14: 864 (1973). (b) Christman D.R., Finn R.D., Karlstrom K.I. and Wolf A.P. Int. J. Appl. Radiat. Isot. 26: 435 (1975).

40. Van Es T. and Staskun B. - J. Chem. Soc. 5775 (1965).

41. Sacks W. - J. Appl. Physiol. 14: 849 (1959).

42. Sacks W. - J. Appl. Physiol. 16: 175 (1961).

43. Sacks W. - J. Appl. Physiol. 20: 117 (1965).

44. Sacks W., Sacks S., Badalamenti A., and Fleischer A. - J. Neuroscience Res. 7: 57 (1982).