Wenn es die leichtere Zugänglichkeit eines e-Wasserstoffatoms ist, welche die Oxydationsgeschwindigkeit von Derivaten von sowohl *Cyclohexan* als von *Tetrahydropyran* bestimmt, müssen β -Verbindungen der Zucker am glycosidischen Kohlenstoffatom ein e-Wasserstoffatom tragen, da β -Verbindungen ja weit schneller oxydiert werden als die anomeren α -Verbindungen. β -D-Glucose und β -Methylglucosid haben demnach eine axiale Hydroxylgruppe oder Methoxylgruppe bei C₁.

Hydrolyse von 1,2-Anhydro-D-Glucose. 1,2-Anhydro-D-Glucose-3,4,6-triacetat wird Brigl-Anhydrid¹¹ genannt. Es enthält einen Äthylenoxyd- oder Epoxyd-Ring. Sowohl in *Cyclohexan*- als in *Tetrahydropyransystemen* haben Epoxydringe einen α - und einen e-Brückenkopf. 1,2-Anhydro-D-Glucose hat bei C₂ einen e-Brückenkopf und muss daher bei C₁ einen α -Brückenkopf haben. Bei lang andauerndem Erhitzen (16–104 St.) auf 100° oder 120° addiert das Brigl-Anhydrid verschiedene Verbindungen unter Bildung von α -Glucosiden (Maltose, α,α -Trehalose, Saccharose). Die Ausbeute ist meistens nur gering. Das Brigl-Anhydrid addiert Alkohole mit ausserordentlicher Leichtigkeit. Die Reaktionsprodukte sind sterisch einheitliche β -D-Glucoside. Aus dem Brigl-Anhydrid werden also einerseits α -Glucoside andererseits β -Glucoside gebildet. Eine der Reaktionen muss unter Umlagerung verlaufen; die Frage ist: Welche? Die Umsetzung mit Carbinol verläuft schon bei Zimmertemperatur in wenigen Stunden quantitativ¹². Unter diesen milden Bedingungen erscheint eine Umlagerung unwahrscheinlich. Es gibt nur eine plausible Erklärung: β -Methyl-D-Glucosid wird ohne Umlagerung gebildet; es hat eine axiale Methoxylgruppe.

Das *Resultat* dieser Untersuchungen steht im Widerspruch mit der heutigen Anschauung. Auf Grund der Übereinstimmung der Ergebnisse mehrerer von einander unabhängigen Methoden möchte ich schon jetzt die Resultate meiner Untersuchungen als "kurze Mitteilung" veröffentlichen. Ein ausführlicher Bericht wird in *Acta Chem. Scand.* erscheinen.

1. Hassel, O. *Tidsskr. Kjemi, Bergvesen, Met.* **3** (1943) 32.
2. Hassel, O. und Ottar, B. *Acta Chem. Scand.* **1** (1947) 929.
3. Jackson, E. L. und Hudson, C. S. *J. Am. Chem. Soc.* **59** (1937) 994.

4. Overend, W. G. und Stacey, M. *Advances in Carbohydrate Chem.* **8** (1953) 94.
5. Barton, D. H. R. *J. Chem. Soc.* **1953** 1035.
6. Magasanik, B., Franzl, R. E. und Chargaff, E. *J. Am. Chem. Soc.* **74** (1952) 2618.
7. Müller, D. *Biochem. Z.* **199** (1928) 136.
8. Keilin, D. und Hartree, E. F. *Biochem. J.* **50** (1952) 331.
9. Isbell, H. S. und Pigman, W. W. *J. Research Natl. Bur. Standards* **18** (1937) 141.
10. Dyfverman, A., Lindberg, B. und Wood, D. *Acta Chem. Scand.* **5** (1951) 253; Lindberg, B. und Wood, D. *Ibid.* **6** (1952) 791.
11. Brigl, P. *Z. physiol. Chem. Hoppe-Seyler* **122** (1922) 245.
12. Hardegger, E. und de Pascual, J. *Helv. Chim. Acta* **47** (1948) 281.

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Column Electrophoresis of Nucleotides from Ribonucleic Acid of Brain Microsomes

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Analysis of the nucleotide composition of ribonucleic acids (RNA) is usually accomplished by ion exchange chromatography or by paper electrophoresis. This latter technique is extremely simple and quick, but it allows the handling of only limited amounts of material. In the present work column electrophoresis has been used, and has proved a good tool for this purpose. It has been employed for analysing the nucleotide composition of RNA from brain microsomes, which are similar to liver and pancreas microsomes in showing a high content of RNA¹.

The separations were performed on a cellulose powder column according to Porath², and with elution beginning before the end of the electrophoretic run for better resolution³. Samples of hydrolysates from about 6 mg RNA were handled in the present experiments, but at least four times higher amounts can be as well separated on the same column (2 cm diameter, 70 cm length). Microsomes were prepared by

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Fig. 1. Electrophoresis of an alkaline hydrolysate of RNA from brain microsomes on a 2×70 cm cellulose column with continuous elution device; 0.05 ammonium formate buffer, pH 3.5; 750 V, 22 mA. (Rubber or plastic joints or tubing were avoided as far as possible, as they were found to release UV absorbing material in the formate buffer.) Elution was started after 22 h, collecting fractions of 5 ml every 20 min for 19 h, fractions of 3 ml every 30 min for the next 20 h, and then fractions of 4 ml every 60 min till the end of the electrophoretic run, which lasted 88 h. From that point, marked by an arrow, elution in the usual way was started. The pattern is expressed as μg present in each fraction.

differential centrifugation from a homogenate of rat brain in 0.25 M sucrose. The fraction sedimenting between $18\,000 \times g$ (15 min) and $105\,000 \times g$ (60 min) was immediately precipitated with trichloroacetic acid (10 % final concentration). The precipitate was washed with trichloroacetic acid and extracted thoroughly with organic solvents. All these operations were performed keeping the temperature as close as possible to 0° , in order to minimize any possible enzymic RNA hydrolysis. The dry precipitate was hydrolysed with 0.3 M KOH at 37° for 18 h. The hydrolysate was neutralized with perchloric acid, and deproteinized by adding more perchloric acid (0.4 M final concentration). The supernatant after centrifugation was neutralized with KOH, and the perchlorate which precipitated overnight at -5° was discarded. This supernatant was used for electrophoresis, either as such or after freeze-drying.

The results of one electrophoretic separation with 0.05 M ammonium formate buffer

pH 3.5 are shown in Fig. 1. The peaks of the four nucleotides, which have been identified from their relative mobilities in comparison with pure compounds, and from their spectra, are completely separated under these conditions. There is in addition an incomplete separation of the two isomers of guanylic acid, as has already been observed by paper electrophoresis at the same pH⁴. The small peaks are probably formed by a small aliquot of oligonucleotides.

The spectrophotometric measurement of the optical densities at $260\text{ m}\mu$ of the fractions collected permitted the calculation of the relative amounts of the four nucleotides, as reported in Table 1. The extinction coefficients given by Beaven *et al.*⁵ were used. The total sum accounts for 90 % of the total nucleotide content of the original hydrolysate, calculated according to Scott *et al.*⁶ If the minor peaks are also taken into account, and their amount approximately calculated with the same factor, then the recovery is 94 %. The table reports for

Table 1. Nucleotide composition (in moles per 100 moles) of RNA of brain microsomes, compared with data on other tissues.

	rat brain micro- somes	cat brain Ref. ⁷	calf thymus micro- somes *Ref. ⁸	rat liver Ref. ⁹
Uridylic acid	19.7	20.6	19.3	20
Guanylic acid	32.5	31.8	33.6	32
Adenylic acid	18.1	21.6	19.1	21
Cytidylic acid	29.7	26.0	28.0	28

* These data have been recalculated, in order to render them comparable.

comparison the only available data on brain RNA⁷. They concern a purified preparation of RNA, obtained from slices of tissue after prolonged incubation. The way of preparation, besides the difference in species, can be the reason for the difference in nucleotide composition. The table reports also some recent data obtained by other authors who have performed, as we have done, direct hydrolysis of the RNA-protein. Although they concern different tissues, they show a close similarity with the present data on brain microsomes.

The fractions corresponding to each peak were pooled and lyophilized twice, in order to remove the ammonium formate. The dry powders, redissolved in a small volume, were used for further analysis. The four recovered nucleotides were submitted to chromatography with various solvents, in order to test their homogeneity. With the solvent *isobutyric acid-ammonia*¹⁰ the uridylic acid gave rise to an additional spot. Its spectrum and chromatographic behaviour correspond to those of the "fifth nucleotide" described by Davis and Allen¹¹.

The same nucleotides were submitted to electrophoresis on paper at pH 2.5. One new spot separated ahead of guanylic acid. When a sample of guanylic acid was submitted to electrophoresis at similar conditions on a small cellulose column, a small faster migrating peak separated, as can be seen in Fig. 2. Its amount, calculated with the same extinction coefficient as guanylic acid, is 2.3 % of the total guanylic acid, and its spectrum is similar to that of the unknown component 1 B obtained by Kemp

Fig. 2. a) Electrophoresis of brain RNA guanylic acid on a 2×18 cm cellulose column, with 0.2 M ammonium formate buffer pH 2.5; 600 V, 70 mA. The elution (5 ml every 5 min) was completed during the electrophoretic run, which lasted 6 h. b) Spectrum of the small, faster migrating peak obtained in a).

and Allen¹² by paper electrophoresis at pH 2.5 of guanylic acid from pancreas RNA.

Paper electrophoresis of the four nucleotides was performed in borate buffer pH 9.2. One slower migrating compound separated from uridylic acid. It was present also in uridylic acid already deprived from the "fifth nucleotide" by chromatography.

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1. Toschi, G. *Exptl. Cell Research*. In press.
2. Porath, J. *Biochim. et Biophys. Acta* **22** (1956) 151.
3. Porath, J., Lindner, E. B. and Jerstedt, S. *Nature* **182** (1958) 744.
4. Davidson, J. N. and Smellie, R. M. S. *Biochem. J.* **52** (1952) 599.

5. Beaven, G. H., Holiday, E. R. and Johnson, E. A. *The nucleic acids* edited by Chargaff, E. and Davidson, J. N., Academic press 1955, p. 493.
6. Scott, J. F., Fraccastoro, A. P. and Taft, E. B. *J. Histochem. and Cytochem.* **4** (1956) 1.
7. De Luca, H. A., Rossiter, R. J. and Strickland, K. P. *Biochem. J.* **55** (1953) 193.
8. Osawa, S., Takata, K. and Hotta, Y. *Biochim. et Biophys. Acta* **28** (1958) 271.
9. Ledig, M., Weill, J. D. and Mandel, P. *Bull. soc. chim. biol.* **40** (1958) 599.
10. Magasanik, B., Vischer, E., Donoger, R., Elson, D. and Chargaff, E. *J. Biol. Chem.* **186** (1950) 37.
11. Davis, F. F. and Allen, F. W. *J. Biol. Chem.* **227** (1957) 907.
12. Kemp, J. W. and Allen, F. W. *Biochim. et Biophys. Acta* **28** (1958) 51.

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Adenosine Triphosphatase in Chloroplasts

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The following two hypotheses are strongly supported by experimental evidence. 1. Light-induced phosphorylation (LIP) is an electron transport phosphorylation ^{1,2}. 2. The ATP-ase * reactions in mitochondria mirror oxidative phosphorylation (OP) reactions, in the reverse direction, going from ATP towards the electron transport chain ³.

Assuming that hypothesis 1 is correct, the question arises as to what extent similar or identical mechanisms are operating in the generation of ATP in OP and in LIP. Assuming that hypothesis 2 is correct, an investigation of the ATP-ase reactions in LIP systems should give information about the possible similarity between the two phosphorylation mechanisms.

Of the two known LIP systems, plant chloroplasts and bacterial chromatophores, the former have been obtained free from respiratory activity. In spinach, the most

investigated plant, this activity is found in the mitochondria, which can be removed. In the photosynthetic bacteria, those fractions which show LIP have not yet been obtained free from the respiratory system. Thus the ATP-ase activity found in these bacteria ⁴⁻⁶ may reflect, at least partly, the OP rather than the LIP system.

A study was made of the ATP-ase activity in isolated spinach chloroplasts. They were prepared ** by the method of Allen, Whatley and Arnon ⁷. Chloroplasts, freed of mitochondria, do not respire, according to these authors. In agreement with this, the oxygen uptake was zero with succinate as substrate and very low with DPNH. Arnon ⁸ has reported that the chloroplasts seem to be rather free of ATP-hydrolyzing enzymes. We obtain, however, a considerable ATP-ase activity in the presence of added Mg^{++} .

The Mg^{++} -dependent ATP-ase activity of whole chloroplasts and chloroplast fragments, prepared as in Ref. ⁸, and the strong inhibition caused by atebtrin are shown in Table 1. Löw ⁹⁻¹¹ has shown that atebtrin and chloropromazine inhibit mitochondrial Mg^{++} -activated ATP-ase and that some other ATP-hydrolyzing enzymes, which are not connected with electron transport systems, are unaffected. 1 mM chloropromazine gave about 85 % inhibition with chloroplast fragments.

Table 1. Mg^{++} -dependent ATP-ase in spinach chloroplasts. Medium: 0.3 ml 0.1 M tris-(hydroxymethyl)aminomethane pH 7.5, 10 μ moles ATP, 8 μ moles $MgCl_2$. Where added: whole chloroplasts or chloroplast fragments equivalent to 0.5 mg chlorophyll, 14 μ moles atebtrin. Final volume 2 ml, 20 min incubation, 30°C. Phosphate was measured according to Lindberg and Ernster ¹².

	μ moles P liberated/h/mg chlorophyll
Whole chloroplasts	9
» » + atebtrin	0.7
Chloroplast fragments	9
» » + atebtrin	0.4

* Abbreviations: P, orthophosphate; ATP, adenosine triphosphate; ATP-ase, adenosine triphosphatase; DPNH, reduced diphosphopyridine nucleotide; M, moles per liter.

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