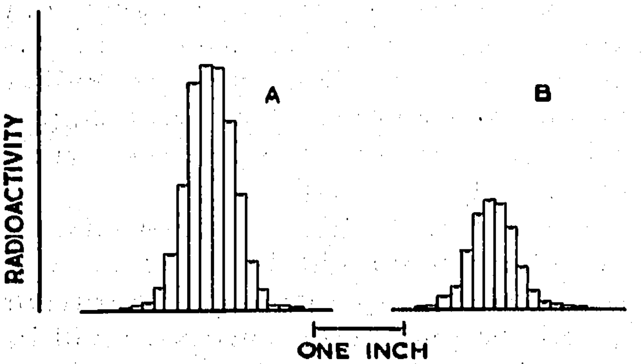
CHROM. 3633

Effects of asymmetrical drying of paper chromatograms on the autoradiography of carbon-14

Little loss of sensitivity occurs when thicker papers are used for paper chromatography and autoradiography. This is partly due to the smaller spot size and consequently increased concentration of radioactive solute. TOMISEK AND JOHNSON[1] deduced from measurements of the surface radiation of ^{14}C compounds spotted on chromatographic papers of different thicknesses that a further contribution to the effect is the migration of solutes to the surfaces of the developed chromatogram during drying. These workers had attempted to show the latter effect directly by producing unequal drying of the 2 surfaces of the paper, but negligible differences were found (ratio of ^{14}C counts on 2 surfaces of Whatman 3MM paper was 1.08 ± 0.12). Earlier, POCCHIARI AND ROSSI[2,3] had demonstrated this type of effect when hot air drying was used on one side of the paper during application of radioactive materials to the origin, but they found that initial differences in count rate of the 2 sides were completely eliminated during chromatographic development of mobile spots, and were only significant in the case of an insoluble compound (glycogen) remaining at the origin. The method of drying of the developed chromatograms was not described.

Some years ago I noticed considerable differences in the count rates of the 2 sides of discs cut from developed paper chromatograms of ^{14}C compounds. The papers had been dried by a hot air blower directed at one side and the discs were counted with an end-window GM counter. This observation has now been re-investigated with [^{14}C]glucose run on paper chromatograms. The solution was applied equally to both sides of the paper at the origin and allowed to dry naturally. Chromatography was then carried out using *sec.*-butanol–acetic acid–water (12:3:5). Replicate papers of different grades were run, and after development some were dried with hot air on one side, while others were allowed to dry naturally at room temperature away from draughts. The papers were scanned with an Ekco Chromatogram Scanner N679B, using a single gas-flow counter to scan each side in turn. Figs. 1 and 2 show that the labelled material becomes considerably more concentrated towards that surface of the paper from which evaporation of the solvent is more rapid, and that the effect is



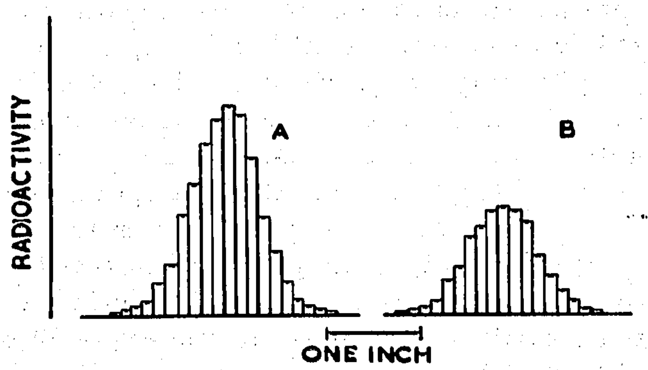


Fig. 1. Scans of the 2 sides of a paper chromatogram of [^{14}C]glucose on Whatman 3MM paper. (A) Side dried with hot air blower; (B) other side. Scanning: 8 in./h, collimator, 0.125 in. Count integrating distance, 0.125 in. Other details as described in text.

Fig. 2. See Fig. 1, but using Whatman No. 4 paper.

seen with both thick and thin papers. No significant difference is seen in papers dried equally on both sides (Fig. 3). Presumably equal drying is the explanation for the finding of equal radioactivity on both sides of the developed papers by POCCHIARI AND ROSSI[2,3].

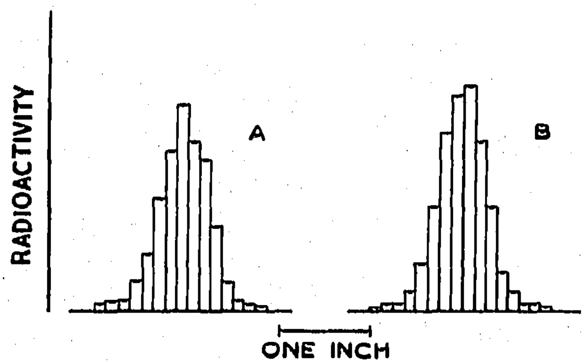


Fig. 3. See Fig. 1, but paper dried equally on both sides in still air.

It is evident from these results that migration of solute towards the surface will, in the case of a paper dried equally on both sides, cause an increased (and equal) concentration at the 2 surfaces and a depletion in the interior of the paper, confirming the deductions of TOMISEK AND JOHNSON[1] already mentioned. Their comment that "unequal drying of the 2 sides of the paper would be negligible under even the most primitive drying arrangements" cannot however be justified.

Since the preferential migration to one surface of an unequally dried paper is in effect chromatography perpendicular to the plane of the paper it seemed possible that it might be more pronounced with solute–solvent combinations having high R_F values. Further experiments were therefore done with other solutes and solvents giving a range of R_F values, the papers being dried by hot air on one side as before and the radioactive areas scanned to give the total counts on each side. The results (Table I) show that there is some correlation between R_F and asymmetrical distribution of radioactivity, the activity on one side of the paper being more than 3 times that on the other side at high R_F values. The intensity of the effect will not therefore be uniform for all spots on a chromatogram, and scanning or autoradiography will not give a quantitatively accurate relationship between the various spots. It is also likely that this will be unavoidable for papers dried equally on both sides, since the chromatographic migration of solutes from the interior to the surfaces will again be dependent on the R_F. Similar considerations apply to the chromatography of non-radioactive substances, where measurements by reflectance densitometry may not give the correct relationship between different spots, even for a uniformly dried paper.

If quantitative measurements are unimportant then the highest sensitivity for qualitative autoradiography can be obtained by deliberately rapid drying of one side of the paper. In the case of thin-layer chromatography, evaporation of the solvent necessarily takes place at the exposed surface, and the maximum sensitivity will be obtained by placing the photographic film against this surface, rather than by stripping off the thin-layer material with the aid of adhesive tape and using the lower surface against the film, as is sometimes done.

The failure of TOMISEK AND JOHNSON[1] directly to demonstrate the effect found

TABLE I

EFFECT OF UNEQUAL DRYING OF 2 SIDES OF PAPER CHROMATOGRAM ON THE SURFACE RADIOACTIVITY OF CARBON-14-LABELLED COMPOUNDS

Labelled compound	*Solvent**	*Paper (Whatman No.)*	R_F	*Ratio* $\frac{\text{counts on heated side}}{\text{counts on other side}}$
Glucose	1	3MM	0.05	1.31
Glucose	2	3MM	0.21	1.75
Glucose	3	4	0.25	1.46
Glucose	4	3MM	0.42	1.67
Glucose	5	3MM	0.80	2.37
Glucose	6	4	0.88	3.87, 3.78**
Glucose	7	3MM	0.91	3.49
Citric acid	8	3MM	0.02	1.18
Citric acid	2	3MM	0.47	1.79
Succinic acid	8	3MM	0.44	1.23
Succinic acid	2	3MM	0.82	3.05

* Solvents: (1) Acetone–water, 92:8; (2) *sec.*-butanol–acetic acid–water, 12:3:5; (3) *n*-butanol; (4) acetone–water, 60:40; (5) acetone–water, 45:55; (6) water; (7) acetone–water, 35:65; (8) phenol–water, 4:1.

** Duplicate scans of same paper to demonstrate reproducibility.

here is possibly related to the rate of evaporation of the solvent from the paper. They apparently did not use heat for drying, and the solvent employed was phenol–water. The present results (Table I) suggest that the differential migration is less with phenol–water than with more volatile solvents, even for an R_F of about 0.4. The overall effect may therefore be due both to the chromatographic properties of the solvent as related to the solute under consideration and to the actual rate of evaporation of the solvent during the drying process used.

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1 A. J. TOMISEK AND B. T. JOHNSON, *J. Chromatog.*, 33 (1968) 329.
2 F. POCCHIARI AND C. ROSSI, *Rc. Ist. sup. Sanità*, 18 (1955) 1421.
3 F. POCCHIARI AND C. ROSSI, *J. Chromatog.*, 5 (1961) 377.

Received May 31st, 1968