lated suspended tumor cells are agglutinated by the sera of animals, not belonging to the Forssman group on account of their containing a specific antigen, but probably related to the heterogenetic Forssman antigen. De Kromme (*4*) has prepared specific antisera by injecting rabbits with carefully washed tumor cells; these antisera react with a precipitation reaction with the lipid tumor antigen excreted with the urine. This observation has been made the base of a diagnostic reaction in the first specific ethers of cholesterol.

As to the chemical nature of the antigen, Kolodziejska and Halber (*5*) and Breinl and Chrobok (*1*) have investigated this by means of complement-fixating tests with the sera of immunized rabbits. Kolodziejska and Halber assume that fatty substances are the specific substance, or that in any case the specific substance is carried with the fatty acids. Breinl and Chrobok locate the antigen in specific ethers of cholesterol.

In the last few years we have renewed our attempts at isolation of this antigen. An example of our recent procedure is offered here. We must confess, however, that so far we have not been able to isolate a substance with a well defined melting point, although we have arrived at a fraction, of which 0.1 μg reacts promptly with precipitation in the form of a ring test when brought in contact with the immune serum.

Perhaps the communication of our procedure may help other investigators to define the nature of the lipid. If so, we believe that an important piece of work will have been performed. In any case, the agreement of polarographic investigations with serological results would be very gratifying.

Case II. Over a period of a week 1100 g of metastatic liver tumor (carcinoma mammae) is extracted with 11 l alcohol, 96% acidified with 10 cc HCl 7 N at 37° C, under frequent stirring.

After filtration, the alcohol is distilled off and the watery residue (*plm.* 1 l) is extracted during 48 hr with a 50% mixture of ether and petrol-ether. The ethereal extract is evaporated to 150 cc and brought to 1 l with acetone. The precipitated phospholipids are filtered off, and the yellow filtrate is evaporated *in vacuo*. The resulting brown oily residue is stirred with 1 l 1% natrium carbonate (pH 9.5). This yellow soap is extracted during 48 hr with ether. The watery solution is brought to pH 3 with 30 cc HCl 25% and the acid solution is extracted again with ether. This ethereal extract is brown.

After evaporating off the ether in nitrogen atmosphere, there remains 8.5 g of a dark brown oil. This is solved in 800 cc alcohol 96% at 70° C. To this solution a boiling clear solution of 5 g lead acetate in alcohol is added; the lead salts of the higher saturated fatty acids are precipitated. After 12 hr standing, these are filtered off on a Büchner funnel. A stream of H_2S is conducted into the clear yellow filtrate containing the soluble lead salts, and the lead sulfide is removed. After evaporation of the yellow filtrate in nitrogen atmosphere the resulting red-brown oil is suspended in 800 cc 12½% ammonia under heating to 80° C. After standing overnight, the ammonia is removed by a stream of nitrogen.

After adding 8 g of barium chloride an orange precipitate is formed, which is washed with water. The precipitate is solved in 80 cc 10% HCl and extracted again with ether. After evaporation (residue: 4½ g) the extract is solved in petrol ether (40–60° C) in order to remove any hydroxy-acids that may be present, and filtered. The solution is agitated with water, dried again, and dissolved in 50 cc dry acetone. The insoluble fraction is filtered off and washed with cold acetone. The brown acetonic solution is cooled for ½ hr at −20° C and the precipitate is rapidly centrifuged in cooled tubes. The precipitate (130 mg) proves highly active in the immunological test (ring reaction with cancer immune serum).

The same treatment is repeated. The rose-colored precipitate is solid at room temperature (26 mg). It is soluble in warm, dry, alcohol-free ethyl acetate: by cooling at 6° C and centrifuging a white precipitate is formed; the ethyl acetate is yellow.

This treatment is repeated four times; now the filtrate is colorless. The precipitate is dried *in vacuo* and becomes slightly brownish thereby. Melting point 100–130° C.

The treatment with barium chloride is repeated, followed by the ethyl acetate procedure described. The resulting precipitate is highly active. One-tenth μg is active in a concentration of 1: 1,500,000.

References

1. Breinl, F., Kindermann, V., and Chrobok, E. *Zeit. f. Immunitätsf.*, 1935, **86**, 270.
2. Carruthers, C. and Suntzeff, V. *Science*, 1948, **108**, 450.
3. ———. *Cancer Res.*, 1949, **9**, 210.
4. de Kromme, L. and Groeneveldt, R. de Bruine. *Vroegtijdige diagnostiek van het carcinoom.* Leiden, Netherlands: Stenfert Kroese, 1939.
5. Kolodziejska, Z. and Halber, W. *Biochem. Z.*, 1930, **225**, 464.
6. Waterman, N. and de Kromme, L. *J. cancer Res.*, 1929, **13**, 60.

A One- and Two-dimensional Paper-Partition Chromatographic Apparatus

Roberta M. Ma and Thomas D. Fontaine

Bureau of Agricultural and Industrial Chemistry, Agricultural Research Center, Beltsville, Maryland

The ascending force of capillary action for chromatographic separation, reported earlier by Williams and Kirby (*1*), has been used in the apparatus to be described here. The apparatus (Fig. 1) consists of a 1-liter graduated cylinder, a No. 13 rubber stopper, some adhesive cellophane tape, and a stainless steel paper holder constructed from two flat and open coils, 60 mm in diameter, and two rods or tubes, 340 and 400 mm in length. Each of the two coils is made from a piece of stainless steel wire or tubing 432 mm in length and 2 mm in diameter. Both ends of the piece of wire or tubing are flattened and fashioned into loops. The longer rod is passed through

the inner loop of the lower coil and is permanently attached to it vertically at a point 25 mm from the lower end. The shorter rod is permanently attached at one end of the outer loop of the lower coil so as to be parallel to

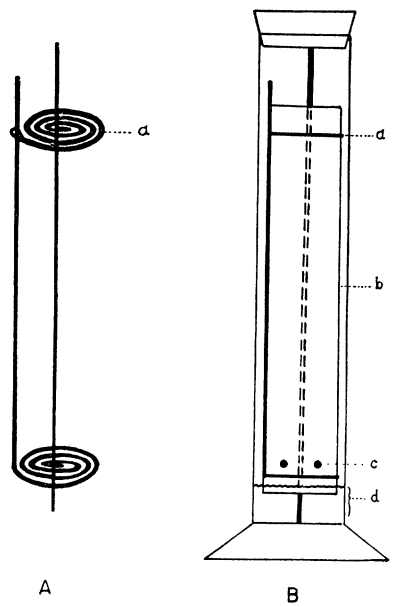

FIG. 1. Chromatographic apparatus. A—Paper holder with adjustable coil (a). B—Assembled apparatus, showing adjustable coil (a), paper (b), position of test spots (c), and liquid volume (d).

the other rod. The two rods are passed through the corresponding loops of the upper coil but are not fastened to them. Since the upper coil can be raised or lowered, it is possible to use sheets of paper of different size, up to 350 mm square. A paper sheet is loaded conveniently by threading it through the coils with a gentle rolling motion.

Chromatograms are usually run on sheets of filter paper 300 to 330 mm long and 230 to 280 mm wide. For one-dimension chromatograms, both the standard and unknown solutions are pipetted onto the paper at a distance of 25 mm from the bottom of the sheet. The liquid usually covers an area 3–5 mm in diameter. After the spots have dried, the paper is rolled into the paper holder. The paper should extend about 10 mm below the lower coil and about 25 mm above the upper coil. The loaded paper holder is quickly placed in a 1-liter cylinder, containing 100 ml or less of solvent, with about 10 mm of the lower edge of the paper in the solvent. The cylinder is closed with the rubber stopper and if it has a lip the hole is sealed with adhesive cellophane tape. The liquid is allowed to ascend by capillary action for a fixed period of time. After expiration of the allotted time, the chromatogram is removed from the cylinder and dried in a stretched horizontal position and then analyzed according to the particular method in use.

For a two-dimension chromatogram, a single spot of test solution is placed in the lower left corner of the sheet of paper, approximately 50 mm from the edges, and the sheet is then placed in the paper holder and treated as described. After expiration of the allotted time, the chromatogram is dried. The sheet is then placed in the paper holder in such a manner that the separated constituents are made to travel upward along the paper at a right angle to the direction from which they moved previously. The final chromatogram is removed, dried, and analyzed.

This apparatus has a number of advantages. It is simple in design, requires small space, and is airtight. The chamber can easily be protected from light if the compounds are light sensitive and construction can be all-glass if conditions demand.

Reference

1. WILLIAMS, R. J. and KIRBY, H. *Science*, 1948, **107**, 481.

Note on an Index of Conformity

Stuart C. Dodd

University of Washington, Seattle

An index to measure the degree of conformity to some norm, or single class interval, of a variable is here proposed. It is an improvement in percentage form of the 4th moment (in sigma units) or Pearson's beta sub-two (taken from an arbitrary origin), which Peters proposed under the label of an "index of institutionalization" (*1*). This index had grown out of studies such as those by Allport on the "J curve of conforming behavior." The formulas for our "index of conformity" (Cfy) and graphs of its behavior are given in Fig. 1. Its derivation is simply that, since the 4th moment varies from unity to infinity, its reciprocal will vary between the limits of 1 to 0. This measures nonconformity so that the complement from unity of this reciprocal is taken to measure degree of conformity. This proportion is multiplied by 100 to express it in familiar percentage units. The origin about which the moment is calculated is the norm or class interval of expected behavior, i.e., any arbitrary origin to which the degree of conformity of the data is to be tested.[1]

$$\text{Cfy} = 100(1 - (\Sigma x^2)^2/N\Sigma x^4)$$

where $x = X - \text{norm}$.

This index measures the degree of concentration in, or dispersion from, one class interval which may be the norm in the social mores, or may be any class interval set up by the analyst as an hypothesis for testing the degree of conformity of the data to it. It measures kurtosis on a scale where 100% is maximum, around 67% is mesokurtic, around 50% is platykurtic, and 0% is negatively

[1] At the limit of complete conformity, when all deviations from the norm are zero, beta sub-two becomes indeterminate, needing evaluation. Aside from the mathematics of this case, its computation gives no trouble, since conformity is evidently maximal and can be so recorded on mere inspection.