CHROMATOGRAPHY

It helps scientists tell one thing from another

by

C. M. STEARNS

TO STUDY THE SUBSTANCES that make up a living system, and that are responsible for its birth, growth, and disease, a biochemist has to be able to separate one chemical compound from the next. That has been one of the big problems of biochemistry from its very beginning. Unfortunately, the chemistry on which life processes are based is so complex, and so sensitive to seemingly insignificant changes, that the separation of pure compounds is often a first-class headache. It is not at all unusual to find two drugs, for instance, whose biological effects are very different—but whose chemical and physical difference is insufficient to allow the chemist to separate one from the other, using only classical methods.

That is where chromatography comes in.

The reason that chromatography is being widely and rapidly adopted by scientists in a whole range of fields is that it *can* often resolve a complicated mixture, even when none of the other chemical and physical levers—the still, the centrifuge, the solvents, the precipitators, and so on—can pry the mixture apart.

Caltech's leading expert on chromatography is Professor L. Zechmeister, who is internationally known for his work in this field. Dr. Zechmeister and his collaborators, as well as the many other scientists at the Institute who are using the chromatographic technique to an ever-increasing extent, are quick to point out that chromatography is not a new idea. It was largely the brainchild of Michael Tswett, a Russian botanist and microbiologist who in 1906 published a pioneer paper on the subject; and the cylinder of adsorbent material that is the hallmark of chromatography is still known as the Tswett column. But Tswett's brainchild lay unnoticed for many years, and only comparatively recently have chemists and biologists begun to develop the seemingly unlimited possibilities of the method.

The method, in all its variations, relies on one physicochemical attribute of all chemical compounds; the capacity for being adsorbed on the surface of solid substances. Inside a small particle (*e.g.*, a gram of powder) the electrical charges are compensated from all directions; but on the surface of the particle there remain unoccupied forces which, under suitable conditions, are able to remove molecules from a solution and hold them firmly. The way adsorption works in a Tswett column is this:

A large glass cylinder is tightly packed with some powder such as calcium carbonate, alumina, or sugar. The solution containing the substance to be separated into its components is poured onto the top of the column. The component with the greatest affinity for the column material (with the greatest "adsorbability") moves down into the column, and soon occupies a top zone on the column; the other compounds migrate further down the column and occupy individual zones there. This resolution process is assisted and made more efficient by washing the column with a suitably selected solvent, which increases the distances between the zones. At the end of this process there is, near the top of the column, a zone of the component with the greatest adsorbability; below this is a second zone, of the component with the second-strongest adsorbability; and so on.

After the separation into zones in the column is completed, the glass tube containing the "chromatogram" is taken from the apparatus and laid on a table; and the

The work of (left to right) Dr. Malcolm Gordon, Francis Haskins, and Dr. Herschel K. Mitchell, of the Biology Division is a good example of the fine distinctions that chromatography makes possible. Using a chromatopile (right foreground), they have been able to break down a mixture of enzymes into several zones, each containing enzymes with slightly different capabilities—an achievement that may add importantly to the understanding of how an enzyme goes about its business of converting raw materials to forms suitable for use by living cells.

column is then forced out of the tube, like a sausage, with a wooden pestle. It is now a simple matter to cut the column into sections, each containing a single component of the original mixture.

Often the separate zones are colored; there may be, for example, a red zone, an orange zone, and so on. In this case one look serves to indicate where to cut the column. When color fails, fluorescence often succeeds; ultraviolet light in a dark room may indicate the location of the zones. Sometimes streaking of the extruded column along its length with a brush will locate invisible and non-fluorescing zones if the brush carries a reagent which develops color with the zone material.

Variations on a theme

That is chromatography in its simplest, and original, form. Two of the modern variations on the original theme increase the versatility of the method markedly. The first of these is the so-called "paper-chromatography," recently introduced mainly by British investigators. A drop of the substance to be analyzed is placed near the corner of a piece of paper and spread out, with the chromatographic technique, first along one side of the sheet. Then the process is repeated in a direction perpendicular to the first. Characteristic spots on the paper result, each spot being formed by a different component of the original substance.

One of the latest developments in chromatography is a cross between the original Tswett column and the paper technique. It was Dr. Herschel K. Mitchell, of Caltech's Biology Division, who invented the "chromatopile."

The chromatopile is a large stack, or pile, of regular, round filter papers (like those used in some kinds of coffee-maker) compressed between metal plates until it is about a foot high. For a typical separation problem, the top few papers are impregnated with the solution of the substance to be resolved, and the solvent is poured down onto the stack of paper through a hole in the top metal plate. As the solvent works down the pile of papers, carrying the unknown material with it, the zones analogous to those of the Tswett column again appear. In many instances it is easy to separate the zones containing different components; in the simplest case, the experimenter can tell the zones apart simply by inspection and then, using only his fingers, can separate the filter-paper discs.

So much for the methods of chromatography. What good have they done?

What chromatography has accomplished

They have made possible a progress in almost all branches of chemistry. At the Institute, for example, much of the work based on chromatography has centered around the carotenoids, an interesting set of colored compounds responsible for such varied effects as the orange of carrots and the red of tomatoes. Some carotenoids are important to human beings because they are converted, in the body, to vitamin A.

More recently, chromatography has been turned to that troublesome class of compounds known, to chemists and biologists, by the name of "cis-trans isomers." It happens that, among the millions of variations on the carbon-hydrogen theme that make up organic chemistry, there are many compounds that have the same number of each kind of atom in their makeup *and* have these atoms hooked together in the same order—and yet are different compounds. The molecules of a compound belonging to this type are able to assume various geometrical forms; they may be straight (rod-like), or bent in the middle (V-form), and so on.

By ordinary chemical standards this is one of the smallest possible differences between two compounds, and two such compounds are accordingly very difficult to tell apart. However, because adsorbability depends on the general shape of a molecule as well as its chemical makeup, chromatography is quite adept at separating the various molecular forms of one and the same compound.

Another class of bothersome compounds to which chromatography is now being applied is that comprising proteins. Proteins, besides being the substances that make up (and hide most of the secrets of) living organisms, are composed of very large molecules, containing many thousands of atoms. There is reason to believe that the very largest protein molecules may even be visible under an electron microscope. But their very size and complexity has made them difficult to handle, from the chemist's standpoint; and he has been particularly handicapped by the fact that many proteins are over-sensitive to his rough handling. In other words, quite often the chemist trying to separate one protein from others damages it hopelessly. It may very well prove possible to devise chromatographic methods which, applied to proteins, will not do such extensive damage to the materials under study, and a good start has already been made in this direction.

The chromatography of amino acids

Much success has already been achieved in the chromatography of the building stones of the proteins, the amino acids. It is now possible to do an amino acid survey of, say, a virus, much more quickly and reliably than by earlier methods. And, using chromatography, a chemist can tell how much of each amino acid occurs in that particular virus, even in cases where the older approach would have led only to rough proportions. That the exact determination of the amino-acid makeup of proteins, whether in germs or in parts of healthy cells, will have extensive effects on the knowledge of life in general and of how to protect it hardly needs to be pointed out.

One final virtue of chromatography deserves a word. It provides an almost ideal purity test. If a solution of a truly pure substance is tested on the Tswett column, only a single zone should appear; on the other hand, contaminants would show up by forming additional (minor) zones.

Because it may appear, from what has been said, that chromatography will solve all the problems of biology and chemistry combined, a few dashes of cold water may be in order. First of all, in any work dealing with huge organic molecules, no method (chromatography included) is going to explain their make-up unless it can work on a pure sample—which is still, more often than not, unavailable. You cannot, in other words, analyze a virus until you have isolated (entirely purified) it; if you have not isolated it, you may be analyzing ten other things at the same time.

How to tell things apart

And finally, chromatography is just one more method of telling things apart. However, it is, as we have seen, extraordinarily selective and efficient. Having a chromatogram to go by is much like having a fingerprint to go by in searching for a criminal, instead of just a description of his overcoat.