References and Notes

1. C. A. Shain, *Australian J. Phys.* **9**, 61 (1956).
2. T. D. Carr, *Astron. J.* **64**, 39 (1959).
3. G. Field, *J. Geophys. Research* **65**, 1661 (1960).
4. V. V. Zheleznıakov, *Astron. Zhur.* **35**, 230 (1958).
5. H. W. Wells, *Proc. I.R.E. (Inst. Radio Engrs.)* **46**, 205 (1958).
6. A. Boischot, R. H. Lee, J. W. Warwick, *Astrophys. J.* **131**, 61 (1960).
7. And in June 1955. See B. F. Burke and K. L. Franklin, *J. Geophys. Research* **60**, 213 (1955); **63**, 807 (1958).
8. M. R. Kundu and F. T. Haddock, *Nature* **186**, 610 (1960).
9. B. R. Burke and K. L. Franklin, *J. Geophys. Research* **63**, 807 (1958).

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Immunological Technique for Protein Isolation

Abstract. Carefully coagulated antibody or antigen-antibody complexes may be used as specific adsorbents for antigen, and the antigen may be released subsequently by increasing the acidity. No cross adsorption appears to take place. The procedure may prove useful for the isolation of tissue-specific proteins (including those in disease states), toxins, or viruses.

Although authoritative texts (*1*) state that denaturation of antibody destroys its combining sites, the earlier results of Kleczkowski (*2*) and Campbell and Cushing (*3*) indicated that such is not universally the case. The studies discussed below have indicated that antibody may be coagulated by at least seven different agents without destroying or masking all of the specific combining sites, and that the coagulum may be used as a specific adsorbent for antigen. The reagents found effective were the following: ethanol, acetone, hydrochloric acid, sulfuric acid, aluminum chloride, chromic chloride, and heat. If the treatment is too rigorous, all sites are destroyed.

Preliminary tests with 12 other denaturing agents failed to leave any specific activity on the antibody; however, more careful control of the conditions might render these other reagents effective. After adsorption of the antigen at about *p*H 7.0 (batch process with continuous stirring for 2 hours), the coagulum may be washed free of nonspecific protein and the antigen desorbed at *p*H 3.0 to 3.5. The coagulum may be re-used at least 12 times. Samples of coagulated antibody directed against ovalbumin, bovine serum albumin, and keyhole-limpet hemocyanin adsorbed and released their own antigens, but no cross adsorption and release could be detected by interfacial ring tests on the eluates.

The coagulated antigen-antibody complex also was effective as an adsorbent for antigen. The optimum conditions for the coagulation of the complex with ethanol were approximately as follows: 34°C, 90 percent ethanol, *p*H 7.0 for 30 minutes. No cross adsorption of antigen appeared to take place. The coagulum could be used as an adsorbent repeatedly. A representative test showed that when 210 μg of ovalbumin nitrogen were added to 1920 μg of antibody nitrogen to obtain the original precipitate, each of the first five adsorption-elution cycles yielded 40 to 50 μg of ovalbumin nitrogen, adsorbed from a large excess of antigen (1350 μg of nitrogen in 10 ml of saline). This yield suggests that about 5 percent of the original combining sites on the antibody were available.

The utility of the process appears to lie in those circumstances in which a protein exists in only one of two otherwise identical solutions. Such conditions are rather common in biology and medicine. One might contrast normal human serum with the serum of an agammaglobulinemic patient, where gamma globulin is present as an extra component in the normal serum, or one might compare a normal serum with one containing Bence Jones protein, where the abnormal Bence Jones protein is the additional component. The examples need not be as dramatic or as simple as the above, for there are many disease conditions, both acquired and hereditary, where normal proteins are lacking or where abnormal ones appear. In some cases only a single protein may be involved, whereas in others there may be several. Given these prerequisite conditions, it should be possible to isolate the additional component, or components, by a process similar to that described below, wherein ovalbumin has been isolated from a synthetic mixture of ovalbumin and dog serum.

A sample of dog serum was divided into two parts, and to one portion ovalbumin was added, to a concentration of 0.85 mg/ml. The ovalbumin–dog serum mixture was injected into rabbits, 1 ml per injection three times a week for 3 weeks. The rabbits were bled on the 7th day after the last injection, and antiserum was prepared. The antiserum had a very high titer for dog serum (the value was not determined) and contained about 0.26 mg of precipitable antibody to ovalbumin per milliliter. The antibody to dog serum was fractionally adsorbed in the following manner: Unadulterated dog serum was added to the antiserum, the mixture was left 2 hours at room temperature and 22 hours at 0° to 5°C, and the precipitate was spun off. Two hundred milliliters of antiserum were used, and the volumes (in milliliters) of dog serum added were as follows: 5, 5, 5, 10, 10, 10, 20, 20, 20. The last two additions produced no further precipitate.

Table 1. Yield and purity of ovalbumin eluted from a mixture containing 1 part of ovalbumin in 68 parts of dog-serum protein.

Elution No.	Ovalbumin (μg N)	Total protein in eluate (μg N)	Purity of ovalbumin (%)
1	70	125	56
3	47	115	41
5	44	83	53
7	34	58	59

The mixture was then analyzed for antibody to ovalbumin by the quantitative precipitin technique, and the calculated quantity of the ovalbumin–dog serum mixture was added to precipitate the ovalbumin-antiovalbumin complex at equivalence. The resulting precipitate was washed five times and then coagulated by treatment with 90-percent ethanol for 30 minutes at 30°C, *p*H 7.0. The coagulum was washed three times at *p*H 3.0 and three times at *p*H 7.0 to remove the coagulating agent and any uncoagulated complex.

Twenty milliliters of the ovalbumin–dog serum mixture were then added, and the solution was stirred for 2 hours at room temperature. This volume of the mixture contained a large excess of ovalbumin nitrogen (about 2700 μg). All unadsorbed protein, as determined by ring-testing the washes against a portion of the original antiserum, was washed off. The ovalbumin was then desorbed at *p*H 3.1 with buffered saline (0.05*M* glycine-HCl buffer) overnight. The coagulum was spun off, and the supernatant was brought to *p*H 7.0 and analyzed for total protein by the biuret method, and for antigen by quantitative precipitin determinations with a known antiovalbumin antiserum. The coagulum was re-used to adsorb more ovalbumin from a further sample of the same mixture.

As indicated in Table 1, the yield of ovalbumin decreased gradually from 70 to 34 μg of ovalbumin nitrogen over the first seven adsorption-elution cycles. This decrement continued through the 12th cycle (26 μg of nitrogen), when the process was terminated. Data beyond the seventh elution are not included because of the unreliability of the biuret determinations at these low protein levels. The purity of the product averaged a little better than 50 percent. The composition of the impurities has not been studied; however, the possibility exists that part or all of the material may be complement. Since the original ovalbumin–dog serum mixture consisted of one part of ovalbumin nitrogen in 68 parts of dog serum ni-

trogen, the results indicate approximately a 35-fold increase in purity. Further increases in yield and purity should be obtainable as more information becomes available about methods of coagulating the complex and of adsorbing and desorbing the antigen.

As contrasted with the usual physical-chemical methods for protein isolation, this procedure is simple and involves comparatively mild environments for the protein, thus reducing denaturation. In those cases where the procedure is applicable, the method is direct, it depends on a biological property of the protein, and it is highly specific. It should separate proteins with different biological properties but of similar physical-chemical properties, where the conventional techniques would fail. The process may prove useful for the isolation of tissue-specific proteins (including those in disease states), viruses, or toxins (*4*).

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References and Notes

1. E. S. West and W. R. Todd, *Textbook of Biochemistry* (Macmillan, New York, 1951), p. 383; K. Landsteiner, *The Specificity of Serological Reactions* (Harvard Univ. Press, Cambridge, Mass., 1945), p. 149.
2. A. Kleczkowski, *Brit. J. Exptl. Pathol.* **22**, 192 (1941).
3. D. H. Campbell and J. E. Cushing, *Science* **102**, 564 (1945).
4. This investigation was supported in part by Public Health Service research grant No. H-3728 from the National Heart Institute, Public Health Service, and in part by the U.S. Air Force Aerospace Medical Center (ATC) School of Aviation Medicine, under contract No. AF 41(657)-320. I am indebted to Norman McDuffie and Virgil Peters for their technical assistance.
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Continuous Elemental Analysis of Organic Compounds in Gas-Chromatographic Effluents

Abstract. The compounds emerging from the gas-chromatographic column are quantitatively converted to a mixture of CO_2 and H_2. These gases are separated by means of an auxiliary column. The ratio of C and H atoms of each substance is deduced from the areas of carbon dioxide and hydrogen peaks.

A simple method for continuous analysis of the carbon and hydrogen content of volatile compounds previously separated by gas chromatography has been developed.

Substances emerging from a gas-liquid partition column are quantitatively oxidized on copper oxide to form carbon dioxide and water (*1*). The water is reduced to hydrogen by finely divided iron (*2*). Carbon dioxide and hydrogen are then separated by means of an auxiliary column, their concentration in effluent gases being determined by a thermoconductivity cell operated at room temperature.

Thus, two separate peaks, one for carbon dioxide and the other for hydrogen, are obtained for each compound, the ratio between areas under the curves depending on the elementary composition of the substance, independent of its weight.

In the case of hydrocarbons the empirical formula may be directly deduced from the ratio of H_2 and CO_2 peak areas, while with oxygenated compounds only the ratio between carbon and hydrogen atoms is obtained.

The gas chromatograph employed is a model B "Fractovap" of the Società Carlo Erba, Milan, Italy, equipped with stainless steel columns and connected to a model 62 electronic integrator.

The column of the apparatus is connected by means of a silicon rubber fitting to a coiled silica tube 60 cm long, having an internal diameter of 6 mm. This tube is filled in the first section with copper oxide and in the second one with reduced iron on inert support and is heated to 725° ± 10°C by means of an electronically controlled furnace.

Combustion gases are first passed through a 4-m auxiliary column packed with acetonylacetone on Celite C 22 to separate CO_2 and H_2, and then analyzed in a thermoconductivity cell. The conversion of a number of volatile organic compounds (containing C, H, and O) to a mixture of carbon dioxide and hydrogen is found to be quantitative throughout a wide range of experimental conditions (that is, carrier gas flow rate, reaction tube temperature, size of the injected sample, and so on).

Figure 1 shows three typical chromatograms obtained from benzene, cyclohexane, and ethyl ether.

The values for the carbon-hydrogen ratio in the empirical formula of analyzed substances, as deduced from CO_2 and H_2 peak areas, are summarized in Table 1; these values compare favorably with the experimentally derived ratios.

Table 1. Carbon-hydrogen ratios in organic compounds as derived from chromatogram peak areas. Standard deviation of listed values in column 4 is more than 3 percent. Benzene was used for calibration.

Substance	Peak areas (cm^2)		Ratio of peak areas (H_2 : CO_2)	Carbon-hydrogen ratio	
	H_2	CO_2		Calculated	Observed
Benzene	634	140	4.53	1.00	1.00
Cyclohexane	515	55.6	9.26	2.00	2.04
Ethyl ether	547	47.9	11.42	2.50	2.52

A small variation in the method's sensitivity—that is, in the ratio of peak areas to sample weight—was observed from day to day. When precise results are required, it is advisable to calibrate the apparatus by means of a standard substance having a known carbon-hydrogen ratio before running a series of analyses.

The proposed method, as compared to the conventional gas-chromatographic technique, offers the remarkable advantage of determining, by means of a thermal conductivity detector, a molecular parameter (such as the ratio of C and H atoms in the empirical formula) which allows, in most cases, direct identification of eluted compounds. Moreover, the detector sensitivity is markedly improved, as long as the katharometer can be operated at low temperature.

From the analytical point of view there is the advantage that separation and carbon-hydrogen ratio determination of such a minute amount as 0.05 mg of an organic substance contained in a complex mixture can be simultaneously performed.

Quantitative combustion, resulting in a mixture of carbon dioxide and hydrogen, of substances separated by gas

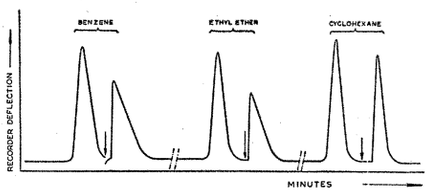

Fig. 1. Analysis of benzene, ethyl ether, and cyclohexane. Main column: 2 m long, internal diameter 6 mm, packed with a mixture of 25 parts by weight of di-*n*-decyl-phthalate on Celite C 22 (40 to 70 mesh). Temperature, 70°C. Auxiliary column: 4 m long, internal diameter 6 mm, packed with a mixture of 28 parts by weight of acetonylacetone on Celite C 22 (40 to 70 mesh). Temperature, 18°C. Carrier gas, nitrogen (flow rate, 0.7 lit./hr). Vertical arrows indicate polarity inversion of potentiometric recorder and sensitivity increase of the thermoconductivity detector. Ratio, 1:4 for benzene and 1:8 for cyclohexane and ethyl ether peaks.