exposure. The subject's hematocrit remained stable, and 24 hours after exposure all of the hematological and clinical chemistry values were normal. No untoward subjective symptoms or objective signs of illness were noted during the exposure period or during the 24-hour period after exposure.

In experiment 2, subjects 1, 2, and 3 were exposed for a 2-hour period to CH_2Cl_2 at a concentration of 986 ± 104 ppm. The three subjects reported the odor of the solvent at about 1000 ppm to be moderately strong, but not particularly objectionable. No eye, nose, or throat irritation was reported. After 1 hour of exposure, two of the subjects reported mild light-headedness which persisted throughout the remainder of the exposure period and cleared within 5 minutes after cessation of the exposure. Exposure to this concentration of CH_2Cl_2 produced alterations in the VER of all three subjects. Details of these findings will be reported elsewhere (*4*).

The COHb saturation increased in all three subjects, rising to a mean value of 10 percent 1 hour after exposure (Fig. 1). Seventeen hours after the exposure period the COHb saturation was still elevated (3.9 percent). During the 24-hour period after exposure the hematocrit for all three subjects remained normal; there was no increase in urinary urobilinogen formation, and all of the clinical chemistry and hematological values were within normal limits.

In experiment 3, subjects 1, 2, and 3 were exposed to two different concentrations of the solvent: 514 ± 9.5 ppm for 1 hour and then 869 ± 12.1 ppm for the second hour. No untoward subjective symptoms occurred during the first hour of exposure. However, within 15 minutes after the vapor concentration was increased to 868 ± 12.1 ppm one of the subjects developed definite light-headedness which persisted throughout the exposure period and for 5 minutes after the exposure period. The VER of all subjects was altered (*4*).

The base-line mean COHb saturation for the subjects prior to exposure was 0.6 percent. The COHb saturation increased continuously during the exposure period and continued to increase during the first few hours after exposure, reaching peak saturations of 8.5, 6.0, and 4.3 percent in the three subjects. The COHb saturation slowly declined after the exposure period but was still elevated above the control value 24 hours after exposure.

During the 24-hour period after exposure, all hematological and clinical chemistry values were within normal limits, and the total urinary urobilinogen excretion during this 24-hour period remained normal.

In experiment 4, subjects 4 through 11 were exposed to CH_2Cl_2 at a concentration of 514 ± 13.9 ppm for 1 hour. During this interval no untoward subjective symptoms or objective signs of illness were noted.

The base-line mean COHb saturation for the group prior to exposure was 1.5 percent. This value rose during the course of the exposure to a mean saturation of 2.6 percent. This increase in COHb saturation continued to 3.4 percent 1 hour after exposure. At 21 hours after exposure the COHb saturation was still slightly elevated, and repeat hematological and clinical chemistry studies at that time revealed no evidence of any increase in red blood cell destruction.

These experiments revealed that the exposure to CH_2Cl_2 vapor in concentrations of 500 to 1000 ppm for 1 to 2 hours promptly initiated the formation of CO in all 11 subjects tested, a result which suggests that this effect may be of universal occurrence in man. Since there was no overt evidence of increased red blood cell destruction, it would appear that the CH_2Cl_2 may be the source of the CO.

Exposure to CH_2Cl_2 vapor for 2 hours in concentrations simulating those encountered during home paint-stripping operations resulted in COHb saturations in excess of those permitted in industry from exposure to CO alone (*5*). Not only was the peak COHb saturation exceeded (the equilibrium COHb saturation after exposure to 50 ppm of CO is 7.9 percent) but the biological half-life of CO, which is approximately 5 hours in a sedentary individual (*6*), was greatly prolonged, thus intensifying the CO exposure.

On the basis of these limited data, it is impossible to predict the COHb saturation that might be reached as a result of repeated 8-hour exposures to the industrial threshold limit value (TLV) of 500 ppm for CH_2Cl_2. The COHb saturation may well be excessive, and we are of the opinion that additional investigations into the effects of CH_2Cl_2 on human beings are necessary so that the TLV for CH_2Cl_2 can be reassessed and, if necessary, readjusted so as to provide an adequate margin of safety.

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References

1. R. D. Stewart, J. E. Peterson, E. D. Baretta, H. C. Dodd, M. J. Hosko, A. A. Herrmann, *Arch. Environ. Health* **21**, 154 (1970).
2. K. Porter and D. H. Volman, *Anal. Chem.* **34**, 748 (1962).
3. R. D. Stewart, H. C. Dodd, D. S. Erley, B. B. Holder, *J. Amer. Med. Ass.* **913**, 1097 (1965).
4. R. D. Stewart, T. N. Fisher, M. J. Hosko, J. E. Peterson, E. D. Baretta, H. C. Dodd, in preparation.
5. *Threshold Limit Values for 1971* (American Conference of Governmental Industrial Hygienists, Cincinnati, 1971).
6. J. E. Peterson and R. D. Stewart, *Arch. Environ. Health* **21**, 165 (1970).

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Electrical Field-Flow Fractionation of Proteins

Abstract. *Protein separation has been achieved by electrical field-flow fractionation, a heretofore unrealized separation technique. Some advantages of this method relative to electrophoresis are the low voltage required, the lack of adverse heating and support effects, and the existence of the method as an elution technique. A comparison of theoretical and experimental retention shows good agreement.*

Field-flow fractionation (FFF) is a separation method in which various applied fields, working in conjunction with cross-sectional flow nonuniformities in a narrow tube, cause the differential migration of molecules and ions (*1*). In electrical field-flow fractionation (EFFF) the applied field is electrical, making this technique applicable in theory to ionic species, particularly charged macromolecules. Here we report the first realization of EFFF separation.

The conceptual basis of EFFF is shown in Fig. 1 (*1, 2*). Laminar flow in a narrow tube establishes a parabolic flow profile having characteristic veloc-

ity differences from point to point in the cross section. The applied electrical field forces the charged species to one wall, this wall being a membrane not permeable to the species. At steady state each species will form an atmosphere near the wall. The mean thickness of the atmosphere, l, is determined by the interplay of the electrical field, which is pushing the charged units toward the wall, and diffusion, which is encouraging their escape. It is given by the equation

$$l = D/\mu E \qquad (1)$$

where D is the diffusion coefficient, μ the mobility, and E the field strength.

Clearly l is different for each species. Species with a large l (zone B in Fig. 1) will be carried forward at a high rate because they extend up into the high-velocity region of parabolic flow. Components with low l (zone A) hug the wall where the downstream velocity is minimal. For this reason they are highly retained.

The theory of FFF (*3*) relates the retention ratio R of a zone to l

$$R = 6(l/w)[\coth (2l/w)^{-1} - 2l/w] \qquad (2)$$

where R is the zone velocity divided by the velocity of an uncharged tracer and w is the width of the flow channel. Components with different l's can be separated by virtue of differences in the retention ratio, R.

It is instructive to compare the potential efficacy of EFFF with that of electrophoresis. In theory a small lateral voltage in EFFF is effectively multiplied by the ratio of column length to width, so that equivalent separation is provided with greatly reduced voltages. Also in theory a very high field strength (but still a low voltage because of the small lateral dimensions) can be used without causing adverse migration patterns due to thermal effects as in electrophoresis. Migration occurs in one phase without a support medium ridding the method of troublesome support interactions. Finally, EFFF is an elution technique, with all attendant advantages in sample detection and collection.

The apparatus used to implement EFFF was constructed with membranes cut from regenerated cellulose dialyzer tubing. The flow channel, formed by clamping a Mylar spacer 0.356 mm thick between two such streched membranes, had a cross section 0.356 mm by 25.4 mm. Platinum wire electrodes were placed above and below, 51 mm apart. Because of the narrowness of the channel compared to the spacing between the electrodes, only about 0.356/51 or 0.7 percent of the applied potential was actually utilized in the achievement of separation. The samples were injected by syringe, and the eluted peaks were detected with a Laboratory Data Control ultraviolet detector.

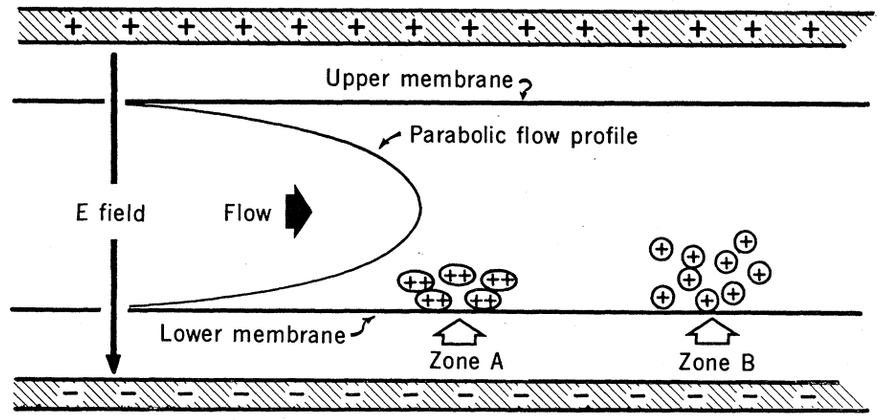

Fig. 1. The electrical field-flow fractionation (EFFF) concept.

Figure 2 shows the separation of three protein mixtures. The separation of albumin and lysozyme (Fig. 2A) and the separation of albumin, hemoglobin, and γ-globulin (Fig. 2B) were carried out in 0.02M solution of sodium acetate adjusted to pH 4.5 with acetic acid. The separation of γ-globulin and albumin (Fig. 2C) was achieved in a 0.02M solution of tris(hydroxymethyl)aminomethane adjusted to pH 8.0 with acetic acid. The values of the field strength, E, reported for these separations are associated with total voltages of 12.5, 15, and 20 volts, respectively, applied across the distance (51 mm) between the electrodes. In all cases, the sample sizes were approximately 50 μg.

The dotted peak in each part of Fig. 2 demonstrates the behavior of one of the separated components in the absence of a field ($E = 0$) under the same flow and buffer conditions used to achieve separation. This peak may be considered the inert marker peak characteristic of unretained components.

Resolution in the above separations would undoubtedly be improved with lower flow rates. Our flow rate, 6.0 ml/hour, was about 75 times larger than the calculated optimum value for maximum resolution.

The order of elution for the components shown in Fig. 2 can be predicted from Eqs. 1 and 2. The precise values of retention time are, however, occasionally at variance with those from the theoretical equations, apparently because of experimental nonidealities. For instance the differential permeability of the membrane to buffer ions of different charge may give rise to electroosmotic phenomena [such as the Bethe-Toropoff effect (*4*)] and an altered field strength.

Equation 1 predicts that a plot of l/w against $1/E$ would yield a straight line of slope $D/\mu w$ through the origin. Figure 3 shows such a plot for albumin at pH 8.0. The theoretical line was calculated with the value 6×10^{-7} cm^2/

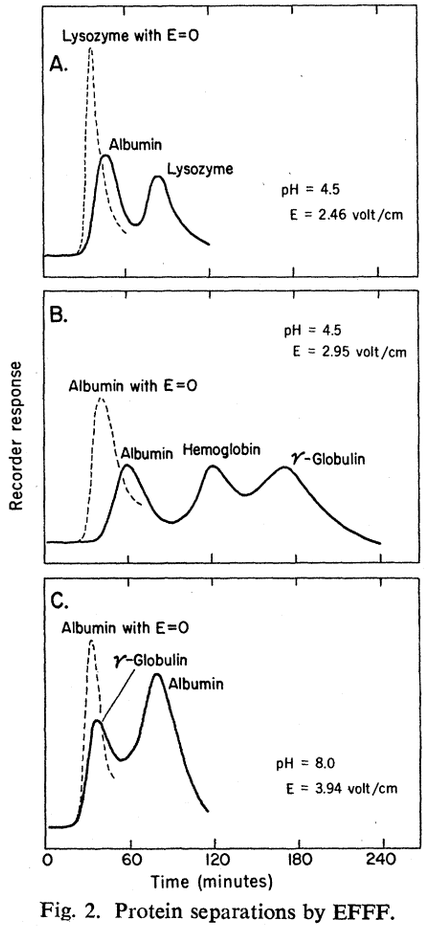

Fig. 2. Protein separations by EFFF.

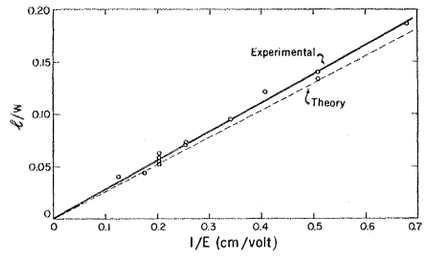

Fig. 3. Linear plot showing the effect of the field strength E on the thickness l of the solute layer.

sec for D (*5*) and -6.5×10^{-5} cm²/sec volt for μ (*6*).

The agreement between theory and experiment in Fig. 3 is exceptionally good. Some results for different systems show a comparable agreement with theory, whereas others are in accord only with the trend of the theoretical curve, and show departure in some important detail. Hemoglobin at *p*H 8.0, for instance, produced a line intercepting the positive ordinate rather than the origin. All proteins at *p*H 4.5 generated linear plots of approximately correct slope but with negative intercepts.

The reasonable agreement between theory and experiment makes it possible to use theory as a tool in tailoring experimental conditions for optimal EFFF separations.

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References and Notes

1. J. C. Giddings, *Separ. Sci.* **1**, 123 (1966).
2. ———, *J. Chem. Phys.* **49**, 81 (1968).
3. M. E. Hovingh, G. H. Thompson, J. C. Giddings, *Anal. Chem.* **42**, 195 (1970).
4. R. Stauffer, in *Physical Methods of Organic Chemistry*, A. Weissberger, Ed. (Interscience, New York, ed. 2, 1956), part 1, p. 65.
5. C. Tanford, *Physical Chemistry of Macromolecules* (Wiley, New York, 1961), pp. 358 and 383.
6. A. Tiselius and P. Flodin, in *Advances in Protein Chemistry*, M. L. Anson *et al.*, Eds. (Academic Press, New York, 1953), vol. 8.
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Induction of Pulmonary Edema and Emphysema in Cattle and Goats with 3-Methylindole

Abstract. *Microorganisms from rumen converted* L-*tryptophan and indoleacetic acid to 3-methylindole in vitro. Oral doses of 3-methylindole caused interstitial pulmonary edema and emphysema in cattle and goats. Intravenous infusion of this metabolite also induced pulmonary disease in cattle. These results demonstrate than an end product of ruminal fermentation of tryptophan can induce acute pulmonary disease in cattle and goats.*

Acute bovine pulmonary emphysema (ABPE) has been recognized as a naturally occurring disease of cattle for over 100 years (*1*). Despite substantial research, the etiology of the disease remains unknown. Our investigations have shown that oral doses of DL-tryptophan (0.57 to 0.70 g per kilogram of body weight) cause interstitial pulmonary edema and emphysema in cattle (*2, 3*). The onset, clinical signs, and pulmonary lesions of the tryptophan-induced disease are similar to those seen in naturally occurring ABPE. Cattle affected by the naturally occurring or experimentally induced disease have labored, irregular breathing with an increased respiration rate; they often die from interstitial pulmonary edema and emphysema within 1 to 7 days after exposure to inducing agents. Necropsy reveals over-inflated lungs, dark red in color and firmer than normal; alveoli are edematous and ruptured, and there is proliferation of alveolar epithelial cells with variable infiltration of neutrophils or eosinophils (*4, 5*).

Only oral doses of DL-tryptophan are effective in causing pulmonary disease even though comparable increases in tryptophan concentrations in plasma are obtained after intraperitoneal injection or intravenous infusion of tryptophan (*3*). These results suggest that a product of tryptophan fermentation in the rumen may be a causative factor in the development of tryptophan-induced pulmonary disease in cattle. Oral doses of tryptophan do not cause pulmonary disease in sheep (*3, 5*) or in goats (*5*). The objective of our experiments was to identify a product of ruminal tryptophan fermentation that is capable of causing pulmonary edema and emphysema in cattle. Identification of this metabolite may be significant in the search for causative agents in pulmonary disease of other species.

We tested the effect of D- and L-tryptophan on the development of pulmonary edema and emphysema in cattle. Three steers given an oral dose of D-tryptophan (0.4 g/kg) and one steer given 0.35 g/kg orally did not develop clinical signs of pulmonary disease. An oral dose of L-tryptophan (0.35 g/kg) caused pulmonary disease in two steers. The results indicate that L-tryptophan is the effective isomer, and we have subsequently used L-tryptophan (0.35 g/kg) for experimental induction of pulmonary disease in cattle. Oral doses of indoleacetic acid also cause pulmonary disease in cattle (*5*). Three of six cows developed clinical signs of pulmonary disease, and two died after being given an oral dose of indoleacetic acid (0.6 g/kg).

Our experiments were designed to identify the products of ruminal fermentation of L-tryptophan, D-tryptophan, and indoleacetic acid. We incubated these compounds (10 mg) in vitro for 8 hours under anaerobic conditions with strained rumen fluid (25 ml) obtained from a fistulated steer fed only hay. The D and L isomers of [^{14}C]tryptophan were isolated from DL-[^{14}C]-tryptophan (uniformly labeled in the benzene ring) by paper chromatography according to the method of Loh and Berg (*6*) except that *N*-butanol was substituted for 2-pentanol in the solvent system. The compounds L-[^{14}C]-tryptophan (0.55 μc), D-[^{14}C]tryptophan (0.35 μc), and [2-^{14}C]indoleacetic acid (1.0 μc) were added to the appropriate flasks of rumen fluid. Fermentation metabolites were separated by ion-exchange chromatography (*7*) and were identified by thin layer chromatography on silica gel plates with three solvent systems: (i) isopropanol, ammonia, water (20 : 1 : 2), (ii) butanol, acetic acid, water (12 : 3 : 5), and (iii) 6 percent benzene/chloroform. Ehrlich reagent (*8*) was used to locate the tryptophan metabolites.

The chief product of L-tryptophan fermentation was 3-methylindole (3MI); indole and indoleacetic acid were also detected as end products. D-Tryptophan was not converted to any of these metabolites under the experimental conditions. Indoleacetic acid was also converted to 3MI by the microorganisms of the rumen. Indole was present in control rumen fluid incubations without added substrate. An Ehrlich-positive metabolite, tentatively identi-