Since there was obviously a flow of electrons from the anode to the cathode, direct measurement of this cathodic depolarization current seemed possible. Two electrodes (each with a surface area of 1.1 cm^2), made from one of the coupons, were encased in lucite and secured by means of a holder that fit over a standard plastic petri dish bottom. After the two electrodes were dropped on the agar surface and secured, the petri dish was placed in a Brewer's jar and the air was replaced by nitrogen. The two electrodes were connected to a very sensitive vacuum tube voltmeter (Hewlett-Packard, model 412A) equipped with a recorder (Esterline-Angus, model AW).

With a very large number of cells (entire surface growth of 3-day-old trypticase soy broth + agar plate) under one electrode (cathode) and none under the other electrode (anode), a sustained current density of about 1 $\mu a/cm^2$ was obtained for a period of about 9 hours. This corresponds to a corrosion rate of about 2.5 mdd (milligrams/dm^2 day) or about 0.00046 ipy (inches per year) with the formula mdd = ipy × 696 × density (*7*) and taking the density of 1010 steel as 7.85. No appreciable current was obtained in the absence of cells under the electrode. The electrode in contact with the cells (cathode) always showed a positive polarity and the anode a negative polarity, a standard dry cell being used as reference.

By use of this technique, it has also been demonstrated that aluminum and aluminum alloys can be cathodically depolarized. Metals more noble than iron and aluminum in the electromotive series, such as tin, zinc, and lead, appeared to be resistant to this type of attack. This may be due to the toxic effects of these metals and their ions on the hydrogen or other electron transport systems in the cells.

It thus appears that the mechanism proposed by the theory does indeed operate, if it can be assumed that sulfate acts in a similar fashion to benzyl viologen at the iron electrode as an electron acceptor. If such an assumption is made, the corrosion rate appears too small to account for the extensive corrosion (attributed to these organisms in nature) entirely by the Wolzogen Kühr theory.

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Acrylamide-Gel Electrophorograms by Mechanical Fractionation: Radioactive Adenovirus Proteins

Abstract. *A mechanical fractionator was developed to produce electrophorograms by extrusion of polyacrylamide gels through a narrow orifice in a continuous, sequential stream. The system permits separation of uniform fractions free of zone distortion. An electrophorogram of radioactive type-2 adenovirus proteins so fractionated gave a pattern in excellent agreement with the pattern obtained by laborious manual sectioning and in agreement with the pattern obtained on a replicate gel stained with Coomassie brilliant blue R250. The adenovirus particle yielded about ten resolvable protein components in unequal amounts. Like picornaviruses, these icosahedral animal viruses have multiple protein components in the viral coat.*

Electrophoresis in polyacrylamide gels (*1*) is a technique for resolving mixtures of macromolecules. It has revealed previously undetected complexity in many types of samples and is especially adaptable to analysis of protein mixtures. The separated components, if not colored, are usually detected by stains or chromogenic reactions. While such methods may be qualitatively adequate, they are quantitatively reliable only when pure reference substances are available for standardization. Isotopic quantitation of the fractions is usually less variable and more direct, and double isotopic labeling, or combined isotopic labeling and staining, permits quite accurate identifications of mixed components. Autoradiographic methods developed for acrylamide gels (*2*) give high resolution but do not permit easy identification of components by double isotopic labeling.

A particularly suitable configuration for gel electrophoresis is a round column through which the samples migrate vertically (*1*). Methods in which gels are sliced have been previously used (*1*) and I now describe a simple device for sectioning gels. I also describe a mechanical fractionator for round gel columns that produces samples suitable for determination of radioactivity by direct counting in planchet and liquid-scintillation counters and for other forms of analysis. Uniform fractions permitting high resolution are produced by continuous extrusion of the gel through a narrow orifice. When this fractionator was applied to production of electrophorograms of radioactive adenovirus proteins, eight to ten components were electrophoretically separable, indicating a degree of complexity not previously reported for the adenovirion (adenovirus particle).

The extrusion system is shown schematically in Fig. 1*A*, and the fractionator and its drive mechanism are shown in Fig. 1, *B* and *C*. Gels to be fractionated were removed from the glass tubes in which electrophoresis was performed, either by rimming with a 20-gauge 8.9-cm needle and syringe filled with a viscous lubricant (noncross-linked polyacrylamide) or by pushing with a tight-fitting plunger. The gels were completely inserted into the stainless steel sleeve (*2* in Fig. 1) and the plunger (*3* in Fig. 1) was inserted partway. With the gel inside, the sleeve-plunger assembly was screwed into the plexiglass block (*1* in Fig. 1) and the plunger was pushed by hand until the end of the gel was seen to be at the tip of the needle valve (*6* in Fig. 1). The assembled fractionator was then placed in the drive mechanism, and 0.05 percent sodium dodecyl sulfate to carry the crushed gel was pumped into the carrier-fluid inlet (*4* in Fig. 1) at the rate of 4 ml/min with a peristalic pump (*3*). The needle valve was adjusted to an opening of about 0.125 mm and the fractionator motor was started. The gel was crushed as it was pressed through the orifice and the small fragments were carried through a flexible tube, attached to the carrier-fluid out-

let (*5* in Fig. 1), into ringed, stainless-steel counting dishes. In these experiments the tubing was directed into another dish every 15 seconds from the time of first emergence of the gel fragments until the fractionator stopped itself. About 55 to 60 fractions resulted from a 6-mm by 10-cm gel. The filled dishes were oven dried at 90° to 100°C and counted in a Nuclear-Chicago gas-flow counter; the background was about 2 counts per minute (cpm).

The fractionator was tested with two different kinds of gel. To test the uniformity of successive fractions, uniformly labeled gels were made by polymerizing HeLa-cell proteins labeled with carbon-14 (*4*) into gel columns, 10 cm long and 6 mm in diameter, in a gel consisting of 5 percent acrylamide, 0.13 percent *N,N*-bismethyleneacrylamide, 0.1*M* sodium phosphate (*p*H 7.2), and 0.1 percent sodium dodecyl sulfate. Polymerization was catalyzed with final concentrations, by volume, of 0.05 percent *N,N,N′,N′*-tetramethylethylene diamine and 0.075 percent ammonium persulfate. The results of fractionation and counting of such a gel are shown by the open circles of Fig. 2. There was fluctuation that, except for a few points, fell within approximately 10 percent of the average value of 490 cpm; this fluctuation was not a result of counting statistics but reflected the variable way in which the gel breaks up and passes through the apparatus. (In actual electrophorograms, as I shall show later, these fluctuations are trivial relative to the differences of several hundred percent between well-separated fractions.) Recovery was 93 percent; that is, the sum of the counts of fractions was 93 percent of the count of the original sample precipitated and counted on a membrane filter. The 7-percent loss in part reflects a small difference in counting efficiency by the two methods.

Another type of gel, designed to test resolving ability, was made by polymerizing a segment of nonradioactive gel, with about 1.5 cm of radioactive gel above it, and another layer of nonradioactive gel to complete the column. The solid line of Fig. 2 shows that the fractionator maintains sharp boundaries in such a synthetic boundary gel, with little skewing or trailing. Transition from one region to another

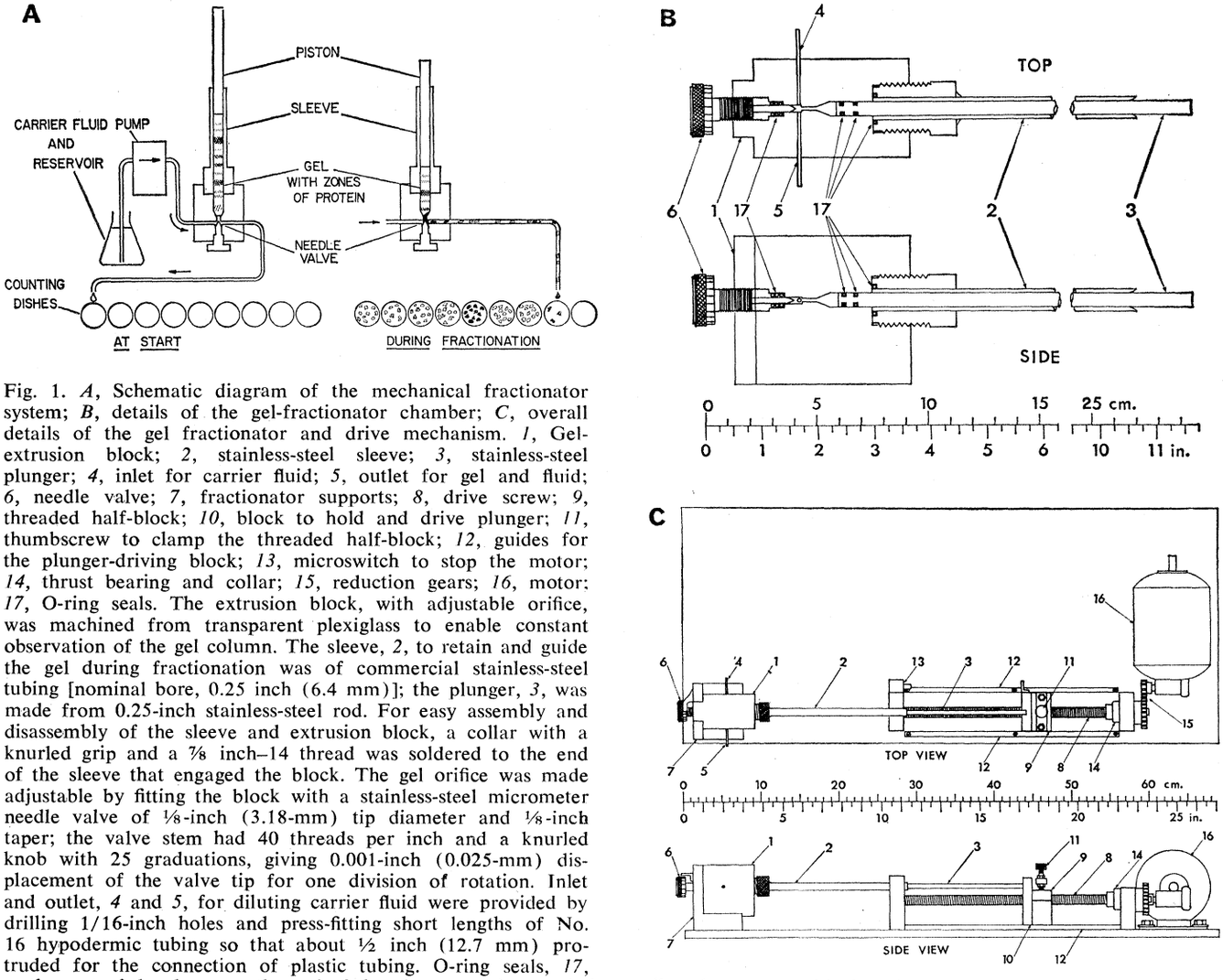

Fig. 1. *A*, Schematic diagram of the mechanical fractionator system; *B*, details of the gel-fractionator chamber; *C*, overall details of the gel fractionator and drive mechanism. *1*, Gel-extrusion block; *2*, stainless-steel sleeve; *3*, stainless-steel plunger; *4*, inlet for carrier fluid; *5*, outlet for gel and fluid; *6*, needle valve; *7*, fractionator supports; *8*, drive screw; *9*, threaded half-block; *10*, block to hold and drive plunger; *11*, thumbscrew to clamp the threaded half-block; *12*, guides for the plunger-driving block; *13*, microswitch to stop the motor; *14*, thrust bearing and collar; *15*, reduction gears; *16*, motor; *17*, O-ring seals. The extrusion block, with adjustable orifice, was machined from transparent plexiglass to enable constant observation of the gel column. The sleeve, *2*, to retain and guide the gel during fractionation was of commercial stainless-steel tubing [nominal bore, 0.25 inch (6.4 mm)]; the plunger, *3*, was made from 0.25-inch stainless-steel rod. For easy assembly and disassembly of the sleeve and extrusion block, a collar with a knurled grip and a ⅞ inch–14 thread was soldered to the end of the sleeve that engaged the block. The gel orifice was made adjustable by fitting the block with a stainless-steel micrometer needle valve of ⅛-inch (3.18-mm) tip diameter and ⅛-inch taper; the valve stem had 40 threads per inch and a knurled knob with 25 graduations, giving 0.001-inch (0.025-mm) displacement of the valve tip for one division of rotation. Inlet and outlet, *4* and *5*, for diluting carrier fluid were provided by drilling 1/16-inch holes and press-fitting short lengths of No. 16 hypodermic tubing so that about ½ inch (12.7 mm) protruded for the connection of plastic tubing. O-ring seals, *17*, at the seat of the sleeve, at the end of the plunger, and around the needle valve prevented leaks. The motor was a 10-rev/min constant speed motor (Bodine type NSY-12R), *16*, with 2 to 1 reduction, *15*, coupled to a ½ inch–20 thread brass drive screw, *8*. A threaded steel half-block, *9*, engaged the drive screw and moved a block, *10*, to drive the plunger. By means of a thumb screw, *11*, and springs, the threaded half-block could be easily released to adjust or reset the position of the block. A switch, *13*, stopped the motor when the plunger was near bottom, in order to prevent damage.

occurs over two or three fractions, corresponding to 3 to 5 mm of gel length—the distance between radioactive bands necessary for maximum resolution. When it is necessary, gel concentration, *p*H, or buffer can be altered to effect the necessary separation.

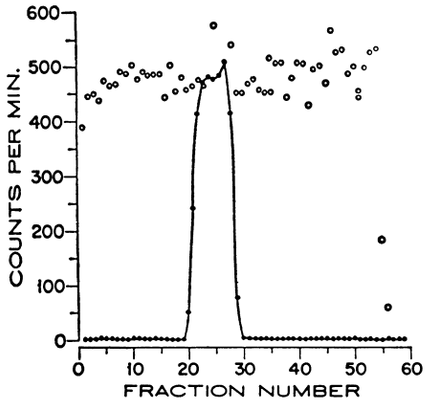

Fig. 2. Fractionation of uniformly labeled and zonally labeled test gels. Open circles, uniformly labeled gel; the average of all fractions was 490 cpm. Solid circles and line; gel having radioactive segment between two nonradioactive segments.

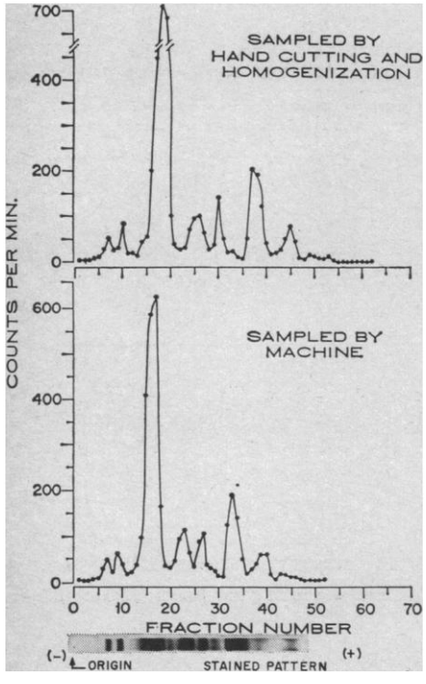

Fig. 3. Comparison of electrophoretic pattern of adenovirus type-2 proteins obtained by manual-sectioning, by automatic fractionating, and by staining with Coomassie blue (top, center, and bottom, respectively). The stained pattern was photographically reduced to obtain agreement with the plot of the machine-fractionated gel; because of different fraction size, the plot of the manually sectioned gel is somewhat longer but has the same proportionate distribution.

Results with adenovirus type-2 structural proteins in a system especially developed for their separation were compared with results obtained by slicing and by staining. Adenovirus type-2 was made radioactive by infecting KB cells with 500 virus particles per cell for 48 hours in Eagle's medium (*6*) containing C^{14} threonine. Virus was purified by the method of Green (*7*), modified by the use of preformed cesium chloride gradients rather than centrifugally formed rubidium chloride gradients. The resultant highly purified radioactive virus was mixed with nonradioactive virus to give a protein concentration of 5 mg/ml and was dialyzed against 0.01*M* sodium phosphate buffer at *p*H 7.2 to remove cesium chloride. Sodium dodecyl sulfate, a powerful virus-dissociating agent (*8*), was added to give a final concentration of 2.0 percent; 2-mercaptoethanol, to 1.0 percent. The opalescent suspension cleared immediately but was allowed to stand at least overnight at room temperature before it was used. No change was detected during storage for from 24 hours to several weeks. Before electrophoresis the sample was diluted to 1 mg/ml and made 25 percent by volume with glycerol. One hundred microliters containing approximately 100 μg of protein and 10,270 cpm was applied to each of three gels, 10 cm long by 6 mm in diameter, of the composition described for test gels. Electrophoresis was for 2 hours at 3 volt/cm. One gel was fixed for 18 hours in 20 percent sulfosalicylic acid, stained in 0.25 percent Coomassie brilliant blue R250 (*9*) for 3 hours, and washed with six 20-ml changes of 7 percent acetic acid to remove excess dye. Another gel was sectioned by hand with a razor blade and a template consisting of two nylon combs held parallel and separated by a spacer 6.35 mm thick. Sections (1.6 mm thick) obtained by cutting through successive, corresponding spaces of the two rows of teeth were homogenized in a Teflon-and-glass homogenizer, transferred to counting dishes, and dried. Homogenization, although laborious, had earlier proved necessary to avoid self-absorption of radioactivity by dried intact slices. (For more energetic isotopes such as P^{32}, intact slices are satisfactory.) The third gel was fractionated mechanically. Figure 3 shows the excellent agreement between the three methods.

Highly purified adenovirions clearly have about ten electrophoretically different kinds of structural proteins. Exposure to sodium dodecyl sulfate and 2-mercaptoethanol, as used by me, dissociates viral structures to their ultimate polypeptide chains without breaking known covalent linkages other than disulfide bonds. The arrangement of these proteins into the shells (*10*) and the 252 capsomeres that have been observed in electron micrographs, and into the relatively few kinds of antigens so far reported (*11*), remains to be elucidated. Preliminary chemical and immunological studies of proteins obtained by electrophoretic fractionation (*12*) suggest greater immunologic complexity than has been reported. Adenoviruses have DNA genomes of about 23×10^6 Daltons (*13*), and therefore have sufficient genetic information for even more than the observed number of proteins. With the same fractionating device and in similar conditions, poliovirus specifies four proteins for virion structure and ten proteins not incorporated in virus particles (*14*); the total number of approximately 14 proteins is consistent with a genome of about 2×10^6 Daltons of RNA. It is probable that adenovirus DNA similarly specifies for a number—perhaps a large number—of nonstructural proteins.

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