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Recently, Korth and Pilchin (1) reported on a simple and rapid method of denaturing biological material with the help of dextran gels. This method is based upon the well-known column chromatography technique in which the stationary phase is a non-synthetic gel type. These dextran gels consist of hydrophilic polysaccharide chains which are interlaced. The degree of interlacing is very constant within the individual gels. They have no active ion groupings, are non-soluble in water, but have a strong affinity for water. The degree of expansion of the gels in water is determined by the interlacing degree of the dextran. The polar character is brought about almost exclusively by the large number of hydroxyl groups of the gel.

The denaturing as well as the fractionation of substances with dextran gels is based mainly upon the differences in their molecular size. When a substance mixture is filtered through a column packed with a dextran gel, the larger molecules migrate faster than those of smaller dimensions. In certain approximation and in the practical application of these methods, the molecular weight can be compared instead of molecular size. Therefore if the difference of the molecular weights of substances is sufficiently great, their complete separation is possible with this gel filtration. In this case, a molecule sieve effect is the main principle and the chromatographic process in its classical
Knowledge of this gel filtration method led us to conduct the following experiments on the use of this technique for desalting of various virus suspensions. The method seemed to us to be particularly useful with respect to the concentration of virus suspensions with sodium sulphate and in the adsorption of the foot and mouth disease virus on aluminium hydroxide and subsequent elution with 1/3 M phosphate buffer, P_i = 7.5 (11). We were looking for a small-scale substitute for the customary dialysis, which often leads to great losses in infectiveness and has certain other disadvantages as well. In order to obtain a broad basis, we examined the behavior of viruses of various origins, differing structuring and characteristics, and suspended in various saline solutions, during gel filtration. In addition to the Newcastle disease virus (NDV) and the virus of pigeon pox (PPV), our experiments dealt mainly with the contagious swine paralysis (polioencephalomyelitis exanthematica swine) virus (Teschenvirus) and the virus of foot and mouth disease (FMD).

Materials and Methods

Virus material: For examination of the smaller types of virus, we used mouse and culture viruses of the HES strain "O₂-Brescia" as well as culture viruses of contagious swine paralysis (Teschener Disease), strain Konratico.

The HES mouse virus came from a 10% extract in culture medium No. 2 (12), which was produced from heart and skeletal muscles of non-born mice of the 35th serial passage of the virus in mice. The HES culture virus used was the 20th serial passage of virus in cultures of swine kidney cells. The virus medium used here was VFl 3a (12). The virus material was stored at -20°C until the start of the experiment. The melted mouse material was always partially purified by diluting it with 50% chloroform (p.A.), shaking for 30 minutes at +4°C and followed by slow centrifugation. The culture liquids were only centrifuged for 10 minutes at 7000 r.p.m. in order to remove cell materials. In both cases,
The Newcastle disease virus and Sendai virus are two of the US viruses from chicken kidney cultures of the first serial passage of the virus.

Harlestone Biozym virus (HBV) -- and influenza virus (IVV) were selected to represent the medium and large size virus types. HBV was available in the form of HLB-C liquid from infected chicken eggs (9th serial passage). IVV in culture virus (9th serial passage in chicken fibroblastic cultures) was used. These viruses were also stored until use at -20°C and after thawing were cleared through slow centrifugation. Further details are mentioned in the text.

Preparation of dextran gel and columns: In our experiments we used as the dextran gel Sephadex G-25 (Pharmacia, Uppsala, Sweden) with a transfection factor of 2.3 grams water/gram dry matter and a grain size (dry) of 50-270 mesh.

The dry Sephadex was suspended in a 1.5% solution of sodium chloride for steeping. After about one hour, we removed very fine grain material through repeated washing and decanting of the gel substance with distilled water. We then removed the small air bubbles clinging to the gel suspended in water through brief evacuation in a suction bottle. Chromatographic columns were then filled with this gel suspension. The column diameter was 1.5 cm and the column length 35-39 cm. In all experiments, we computed from the column diameter (d) and the column length (L) which the Sephadex gel filled, the total gel volume \( V_t = \frac{\pi d^2 L}{4} \).

This value was controlled by measuring the volume of the column filled only with distilled water. In the main experiments, the computed and the measured total gel volume was \( V_t = 62-69 \) milliliter. In comparison tests with HBV, TVV and IAV viruses, we also used smaller columns with a value of \( V_t = 25 \) milliliter. We determined the empty volume (\( V_0 \)) of the columns in prior tests with hemoglobin, which, as a high-molecular protein (molecular weight 68,000), reacts indifferently to the Sephadex gel. Because of its brown-red color, hemoglobin is also a good indicator for the elution of the virus.

The hemoglobin was dissolved to 0.1% in physiological, m/90 phosphate-buffered Sodium chloride solution, \( \rho_s = 7.6 \) (phys NaCl-solution). After slow centrifugation, we brought 5 milliliter of the clear 0.1% hemoglobin solution drop-wise into a column packed with gel, which had been well washed with NaCl solution. After the hemoglobin solution had set, we eluted the blood pigmentation material and took off the eluent in fractions of 3 milliliter. These fractions were then measured individually in an ultraviolet spectrophotometer at 280 millimicron. If the extinctions are plotted graphically to the elution volume in a graph, then
In the virus concentration of the medium, we obtained a flow velocity of 1 to 2 cm per minute. We collected the elution in fractions of 5 to 10 milliliters. All experiments were conducted at room temperature.

Disinfection of gel: The gel was filled with sterile, chloroform-sterilized water. On the day preceding an experiment, the gel was cut out of the gel quantitatively using sterile forceps and saline solutions, taking precautions for sterility. The gel was located at the lower end of our chromatographic column so that it could receive the fraction primary in a sterile condition. By observing sterile precautions, it was no difficulty to achieve a germ-free work.

We disinfected the Sephadex column following an experiment with virus using a 5% Formalin solution which we allowed to remain in the column over night.

Determining degree of infectiveness: We determined the infectiveness of the virus through intraperitoneal injection of 0.5-day-old mice. The Teschen virus was titrated in swine kidney cultures, NDV and TPV in incubated chicken eggs by injection into the allantoic cavity or the chorioallantoic membrane. All titrations were conducted in dilutions in stages of potentials of 10. We computed the titers according to Behrens and Köhler (13). They are based upon 0.1 milliliter of the starting material described under "experiments and results" (ID50 = Lico-ID50; MLD50 = Cultur-ID50; EID50 = EGG-ID50).

Analytical determination: The ammonium sulphate in the individual fractions was determined by distillation in a half micro-Kjeldahl apparatus. Titration was determined with n/70 hydrochloric acid in boric acid.

We performed the phosphate determination according to the molybdate method of Fiske and Subbarow (14), but used ascorbic acid as the reduction medium.
The two experiments MSE-1a and Teschen-3a are graphically portrayed in Illustration 1. The separation of the virus components from the saline gradients can be clearly seen in the elution diagram. The initial value of ID50 is reached in one fraction for each of the two types of virus. If the virus titer of the individual fractions is compared to the final volume, it can be seen that no loss of virus occurred in the desalting. The agreement between the two tests, which was also shown in numerous other tests, is clear. The peak of the virus titer is also identical.
In order to determine the effect of sedimentation of T-even type viruses and T-even inulin virus were no different, but since the lipid-soluble nature of large viruses also behave indifferently to Sephadex gel the small one were also identical in this respect, we examined the T-even virus with 10% and 14% in additional tests. For this we used columns of linear sucrose (V_t = 25 ml). This time the pre-treatment and elution of the gel was done in distilled water which was brought to a pH value of 7.4 with a few drops of 1/10 n KOH. The saline conditions, which have the same electrolyte mixture (cultural medium and egg liquids), were determined through conductivity measurements of the individual solutions.

Illustration 3 shows the results of these comparison tests. All three types of virus have the same elution volume. Their yields are identical and no loss of viruses occurred during the elution. This demonstrated that the desalting is independent of the virus size and virus structure.
Discussion

Gel filtration with Sephadex as the carrier medium was shown to be superior to the mincing technique in all cases of substances of varying molecular size. This has been demonstrated conclusively by numerous publications (1-10). Moreover, the mixing of virus suspensions without loss of infectivity is not possible in most cases by means of the usual dialysis in colloidal membranes, particularly in the case of very labile viruses such as that of HSV. Furthermore, it is time consuming and not suitable for small volumes.

Gel filtration with Sephadex G 25 as the carrier medium proved to be very practical in these cases and offered other possible advantages in other processes.

We have shown with our experiments that such labile viruses as HSV can be desalted very well and without loss of infectivity. Other types of virus, such as the Touschen virus, Newcastle disease virus and the virus of pigeon pox retained their full activity during desalting. Since the elution volume of these viruses (Illustration 3) are equal to each other and also hemoglobin as determined in pre-tests, it can be concluded that Sephadex gel G 25 does not react with the viruses. The peak of the elution volume for each virus component corresponds to the empty volume $V_0$. The HSV and the Touschen viruses are among the smallest presently known viruses whereas NAV and TPV vary from those first name in molecular weight by a factor of about $10^2 - 10^3$. Furthermore, the chemical composition of NAV and TPV is much more complex than that of HSV and Touschen virus. In that we examined widely differing viruses, it can be concluded that the gel filtration technique can be used for the desalting of all types of virus, irregardless of their molecular size and chemical structure.

One additional advantage of this method is the rapid and exact
Our results with viruses and particulate matter were not considered for desalting virions, such as in the saline extraction of the virus nucleic acid and protein. In saline extraction, the advantage of the virus nucleic acid can be enhanced, which are used in volume calculation and process described.

A minor dilution effect occurs, which results in the loss of the particles from the Sephadex gel. In other words, we observe a dilution effect between virus and gel when the virus is mixed with the gel. The diffusion of the virus particles in the gel is not significant. In the most unfavorable case, a dilution factor of 1:1 obtain, and this usually falls within the theoretical limits of the gel the infectiousness used. Our experiments also showed that this dilution factor is unimportant, for in a dialysis in saline solution, particularly with high saline concentration at the dialysis buffer, considerable increase in the volume of the dialysate occurs. The main increase in gel filtration, this increase in volume is independent of the saline concentration and depends solely upon the gel volume. It would seldom exceed the dilution factor of 1:2.

It was seen, as we reported earlier (16), that a saline solution saturated with chloroform shows bactericidal effects. At that time, we determined that vegetative forms of bacteria, molds and yeasts were killed with certainty in 1-3 hours. Bacteria spores are very resistant against chloroform, however. Since pathogenic spore formations very seldom occur, the treatment of Sephadex gel with chloroform-saturated saline solution has sufficed. Also, as mentioned before, we work under antibiotic protection.

The 5% Formalin solution which serves to disinfect the columns after an experiment does not change the characteristics of the gel in any way. It can also be quantitatively eluted. We have conducted more than twenty experiments on such disinfected columns and have always obtained
Virus studies on a variety of plants and animals have been made, especially with the use of Newcastle disease virus. A virus with reactivity similar to that of Newcastle disease virus was observed in these virus-cultured materials.


(Notes: Hans Helma Ludwig and Hans Bruno Retzel extend our thanks for their technical support in the conduct of the experiments.)
Illustration 2. Elution diagram of MKS and Teschen culture viruses in 1/3 m phosphate buffer, pH = 7.5.

Illustration 3. Elution diagram of Newcastle disease, pigeon pox and MKS virus from physiological environments with distilled water, pH = 7.6.

Legend: 
- Virusgehalt/Fraktion = Virus content/fraction
- Leitfähigkeit = Conductivity

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Illustration 1. Elution diagram of MKS mouse virus and Teschen culture virus in ~15% ammonium sulphate solution (experiments 1 a and 3 a).

Legend: A-Wert = initial value
        Fraktion = fraction
        für = for