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A NOVEL VIRUS REMOVAL FILTER AND ITS APPLICATION

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SUMMARY

• *The novel virus removal membrane BMMand the commercially available filter* ^{PLANOVAR} *based on this system provide results both reproducible and predictable for virus removal based on a sieving mechanism. The BMMshows good removability of most viruses, including human immunodeficiency virus, hepatitis B and C and other microorganisms. In addition, immune complexes can be removed efficiently with a good protein permeability. Although BMMhas already been shown to be effective in the field of drug manufacture, the combination of virus removal using BMM with virus inactivation offers opportunities for the wider clinical application of BMM, including blood transfusion.*

INTRODUCTION

• Recently appearing infections such as human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), hepatitis C virus (HCV) and agent of the bovine spongiform encephalophaty (BSE) have given rise to new epidemics and generated the social demands to develop strategies against these infections.

On the other hand, progress in biotechnology has accelerated the development of novel biodrugs. In order to protect patients from infections caused by the injection of the biodrugs contaminated by microorganisms, guidelines have been completed in many countries such as the USA, European Union member states and Japan (1-3).

With this advancement, it has also become necessary to develop a new technology for reducing and/or concentrating microorganisms arbitrarily. The virus removal membrane, named BMM, has been demonstrated to remove particles based mainly on their size. The BMM is fabricated from cuprammonium regenerated cellulose and shaped into the form of a hollow fiber. This membrane has two specific characteristics of reproducibility and predictability in virus removal. These two characteristics are prerequisite conditions for validation of virus removal in the biodrug purification process (4).

Then this membrane is employed to decrease exogenous particles, such as microorganisms and immune complexes from drugs or the body fluid of patients reduction of the particles can be assured. About sixty years ago, viruses were regarded as "filtrable" microbes (5). Novel technology to remove particles selectively without damage or loss of valuable bioactive substances, such as antibodies or antigen proteins, may play an important role in the strategy against viruses. In this review, recent topics relating to the virus removal membrane and its application will be discussed.

Selected abbreviations

AIDS - acquired immunodeficiency syndrome
BSE - bovine spongiform encephalophaty
CJD - Creutzfeldt-Jakob's disease
HBV - hepatitis B virus
HCV - hepatitis C virus
HIV - human immunodeficiency virus
JEV - Japanese encephalitis virus
MULV - murine leukemia virus

ADVANTAGE OF VIRUS REMOVAL COMPARED WITH VIRUS IMAGINATION

• The main objective of drug administration against virus infections is to reduce the infectivity of viruses in a patient by inactivating the target viruses and/or by inhibiting the growth or generation of progeny viruses through biochemical reaction with the drugs. The side effects of the drugs may arise from injection of the exogenous bioactive substance and from the immune complexes of degraded viruses which have been degenerated by the inactivation.

On the other hand, viras removal decreases the concentration of the virus resulting in viral infectivity diminution. The side effects caused by the removal procedure can be minimized through the employment of optimized conditions related to the absence of any additional substance. The only unsolved problem has been the credibility of the procedure. The validation membrane overcomes this problem completely. , ;

WHAT CAN BE EXPECTED IN THE BIOMEDICAL FIELD WHEN A VALIDATION MEMBRANE IS EMPLOYED?

• Expectations of a validation membrane can be divided into two categories: prevention and treatment of virus infections. The former expectation may be achieved by removing the virus from the drag injection solution (especially with biodrugs) during purification in the manufacturing process. This expectation can be extended to the removal of immune complexes in addition to the viras from human plasma without loss of antibody proteins. The latter expectation is based on our strategy of reducing the viras titer in the blood of affected individuals using this membrane. In HIV-infected patients, aggressive viral replication seems to occur even before the development of full-blown AIDS and this viras replication may play a very important role in the pathogenesis of HI V-1. In this case, some beneficial effect can be expected from reduction of the extent of viremia by the membrane filter. Although several points are yet to be clarified, we believe that this will be an interesting challenge in the application of our validation membrane.

AIDS VIRUS REMOVABILITY

• The size of HIV-1 is approx. 100 nni, which allows the virus to be removed easily with the validation membrane. Fig. 1 shows the dependence of the viras logarithmic rejection coefficient <!> defined by equation 1 on the mean pore size $2r_f$. The solution employed was fresh frozen plasma (FFP) and HIV culture supernatant and dead end filtration was performed under a constant transmembrane pressure (AP) of 200 mmHg (7).

$$\Phi = \log 10 (N_0/N_f) \quad (1)$$

where N_O and N_f are the infectivities of viruses in the original solution and its filtrate, respectively. Here, the arrow indicates that the viras concentration in the filtrate is less than the detectable level of 3.4 PFU/ml. The filtrates obtained through filtration with BMM ranging between 10 nm and 105 nm in mean pore size show no infectious particles. Decrease in viras infectivity was due to reduction of the viras concentration, but not to the inactivation resulting from the filtration.

Fig.2 demonstrates transmission electron micrographs of the ultrathin section of the BMM50 after filtration of the culture supernatant of HIV-1 (6). The virions are distributed within the region ranging from the inner surface to a site 5 *im* in from the inner wall surface if the hollow fiber indicating that the virions have been entrapped by the sieving mechanism and that milty-step filtration was achieved within the thickness of the hollow fiber wall. The frequency distribution of the virions caught in BMM has been represented empirically by the linear relationship between their number and this relationship has been confirmed by the theoretical approach (8). When extrapolated to the outer surface, a <& value of BMM50 of more than twenty was obtained (7). This extrapolated value was also consistent with the value obtained by extrapolation of the linear relationship between and the mean size of viras 2v (Fig.3) (9). This figure shows the dependence of O on 2v for the various virions, which were confirmed to be molecularly dispersed without forming aggregates. Fig.3 indicates that knowledge of thevirion size allows the removability of the viras in question by the BMM to be predicted. This predictability of viras removal is one of the conditions that are mandatory for a validation membrane (4).

Table 1 shows example of permeability of the antigen of P24 for HIV-1 (6). All antigens composing an HIV virion whose molecular weight is less than 10^5 can pass through the BMM, moreover, antibodies such as IgG and IgM and/or monoclonal antibodies can permeate completely through the BMM when

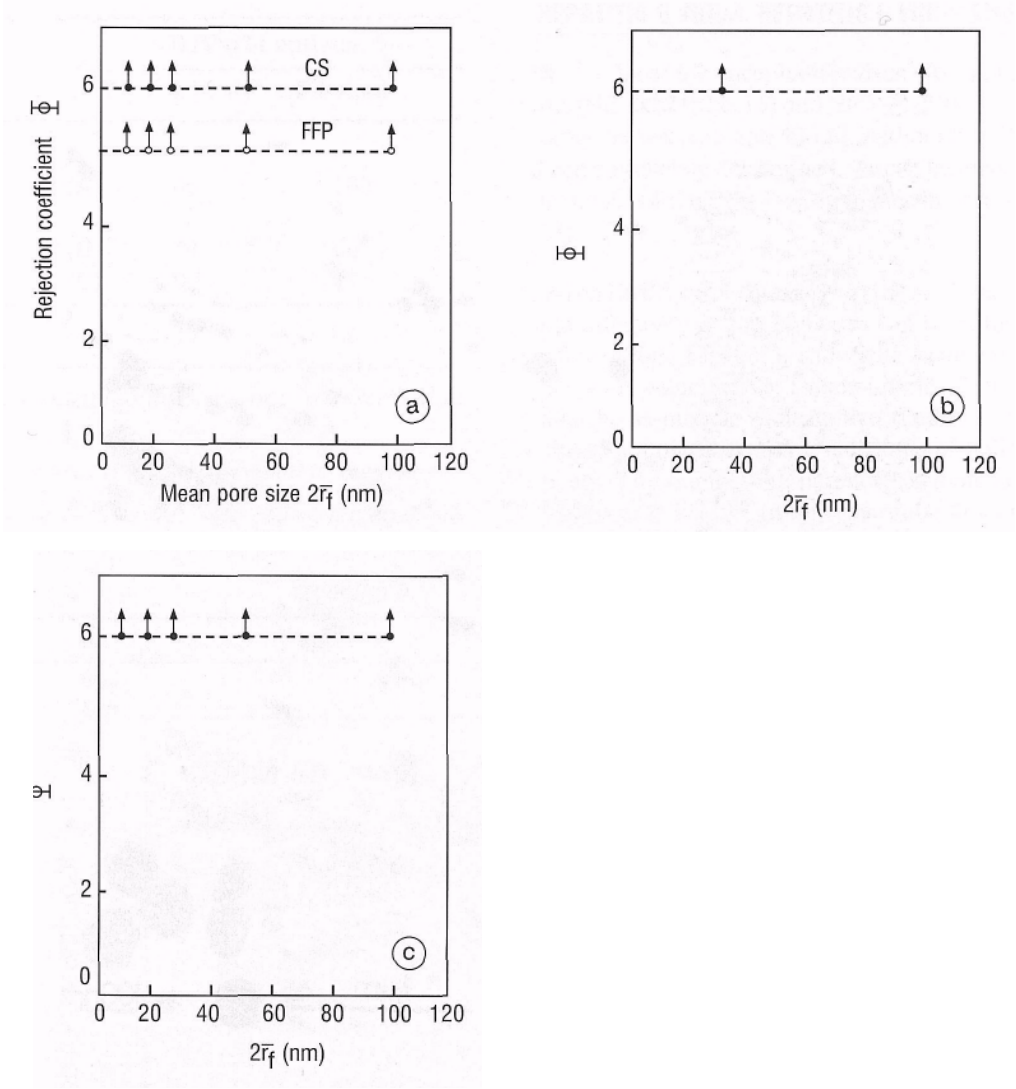

Figure 1. *Dependence of logarithmic rejection coefficient 0 ofHIVon mean pore size 2rfor BMM under filtration condidions. Dead-end, i. e. perpendicular, filtration under constant transmembrane pressure AP of 200 mmffg (a). Dead-end filtration under the constant filtration rate ofL/mm-.min (b). Tangential (parallel) filtration oj AP=200 mmHg and the shear rate of 2000 sec^{-1} at the membrane surface (c). The symbols ofCS and FFP (a) indicate that the solutions dispersing HIV are the culture supernatant and the melt of fresh frozen plasma, respectively. The arrows on the data points indicate that the concentration of HIV in a filtrate is less than the detectable level. From Ref7.*

the mean pore size is more than 15 nm as is shown in the latter section.

When a BMM with a mean pore size of less than 100 nm is employed in the manufacturing process of biodrugs or in the preparation of human plasma, HIV infection caused by transfusion can be prevented. On the other hand, as suggested previously, it may be possible for the BMM to be used for therapeutic purposes in AIDS patients by inclusion of a BMM filtration process in extracorporeal circulation for plasma perfusiori and by the injection of immunoglobulin prepared from pooled plasma of HIV carriers (8), i.e. as immunotherapy (10). This extracorporeal treatment may be effective based on the premise that the ratio between the concentration of the antibody and that of the viruses plays an important role in the actual manifestation of the disease.

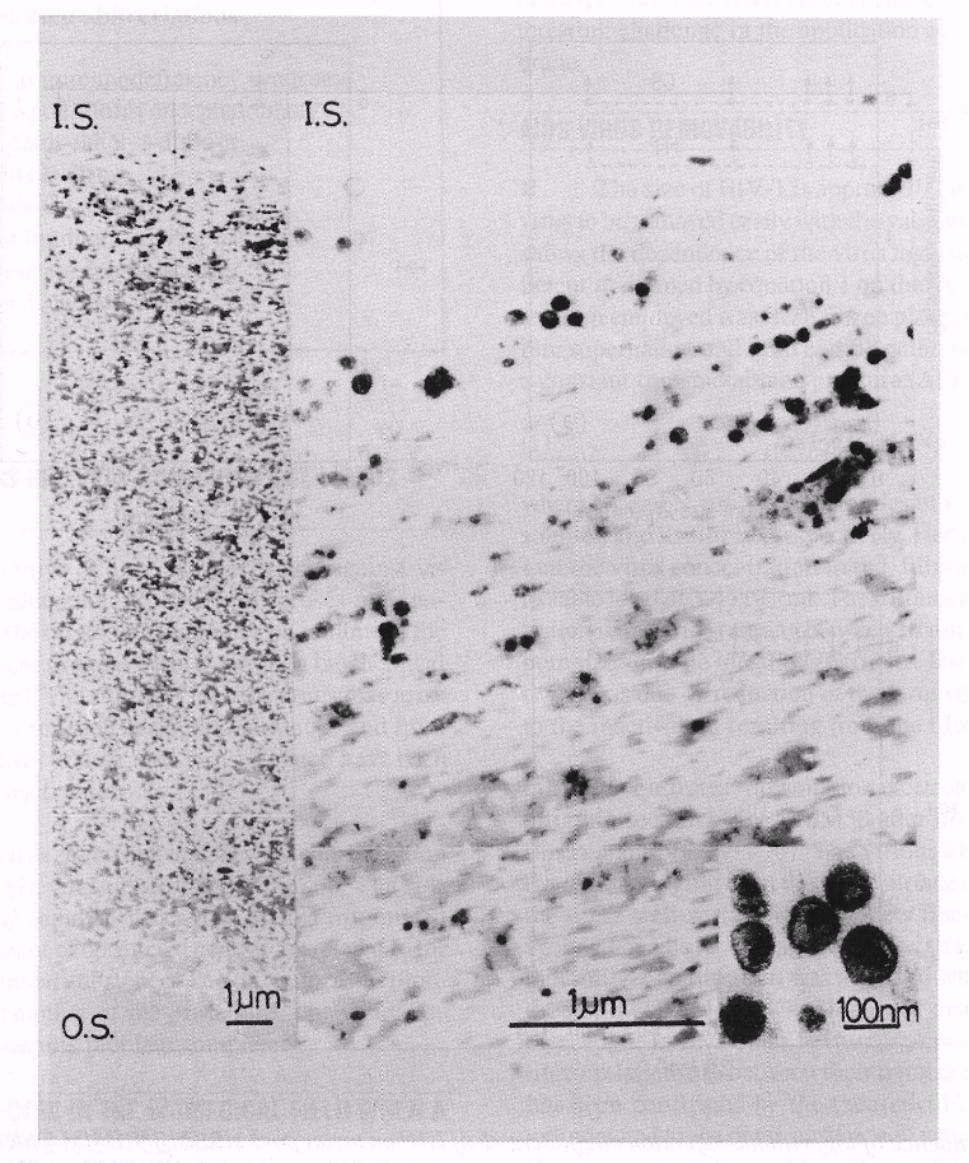

***Figure** 2. Transmission electron micrographs ofBMMSO after the filtration of the supernatant of culture medium containing HIV. Cross-section view with the remaining HIV virions caught in the BALM. The white region corresponds to the source material ofBMM, cellulose, and the black spots located at near the lumen correspond to HIV virions (a). An enlarged micrograph (b). Many mature HIV particles are localized in the narrow space restricted to an area of approximately 5 mm in depth from the luminal surface (a). High magnification of HIV virions caught by BMM (c). I.S. - inner surface ofBMM, O.S. - outer surface of BMM. From Ref 6.* …… ..

Gene therapy has been developed recently using various viruses as vectors and this has led to a need for new technology for virus concentration. The virus removal filter was developed to meet this requirement. More specifically, when the PLANOVA^R filter is employed for virus concentration, concentrated viruses can be obtained easily without significant loss of activity. An ecotropic murine leukemia virus (MULV) and HIV were concentrated 10 to 30 fold from 500-1000 ml of culture supernatant using PLANOVA^R with the filtration area of 0.03 m^2 under the total live virus recovery rate of approx. 50 % (12). PLANOVA^R has been manufactured under more stringent quality control, such as the integrity test for each filter, than in the case of BMM.

Table 1. *Filtr-ability ofHIVp24for BMM50*

Fraction[a]		HIV p24 antigen, %	
		Filtrate[b]	Filtrand
Ultracentrifugation			
Before	(A)	66	100[c]
After			
Supernatant	(B)	54	60
Pallet	(C)	0.3	41
Loss[d]		11.7	-1

" Fractation by ultracentrifugation was done as described in the text.

Filtration procedure with BMM is described in the text.

The concentration ofp24 infiltrandwas 183 ng/ml. The limit of detection of this method is lOOpg/ml.

" Loss = A - (B + C) *From Ref6.*

REMOVABILITY OF JAPANESE ENCEPHALITIS VIRUS, HEPATITIS B VIRUS, HEPATITIS C VIRUS AND OTHER VIRUSES

• Japanese encephalitis virus (JEV) (13), hepatitis B virus (HBV)(11, 14,15)andHCV(4,19) are similar in size and range between 35 and 43 nm. Although HCV size has not yet been completely determined, we can evaluate the size from the <& values of the BMM using the mean pore size of 35 nm (16-

When BMM with a mean pore size of 3 5 nm (BMM3 5) is used, the infectivity of these viruses can be reduced to over the O value of four. Fig.4 (11) shows the mean pore size dependence of the O value for the Dane particle of HB V. The S-module and the M-module indicate that the filters are composed of a single hollow fiber and a multitude of hollow fibers, respectively. The numbers in parentheses indicate the effective filtration area in cm^2. In this figure the <D value decreases with increasing effective filtration area and this tendency is not unexpected from the theoretical viewpoint. The validation membrane should not exhibit this tendency and should show

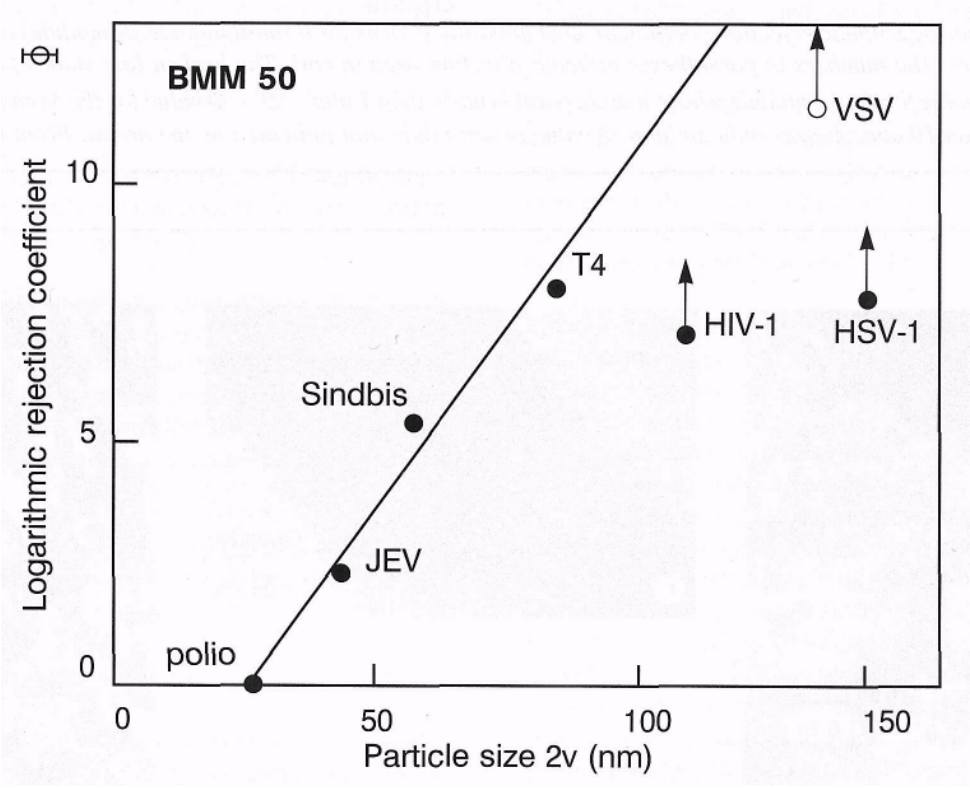

Figure 3. *Dependence of the virus logarithmic rejection coefficient 0 on virion size 2v for BMM50. The viruses employed are present inamonodispersedstate. Abbreviations: polio -polio virus, JEV-Japanese encephalitis virus, HIV-1 - human immunodeficiency virus-1, VSV - vesicular stomatitis virus, HSV-1 - herpes simplex virus-1.*

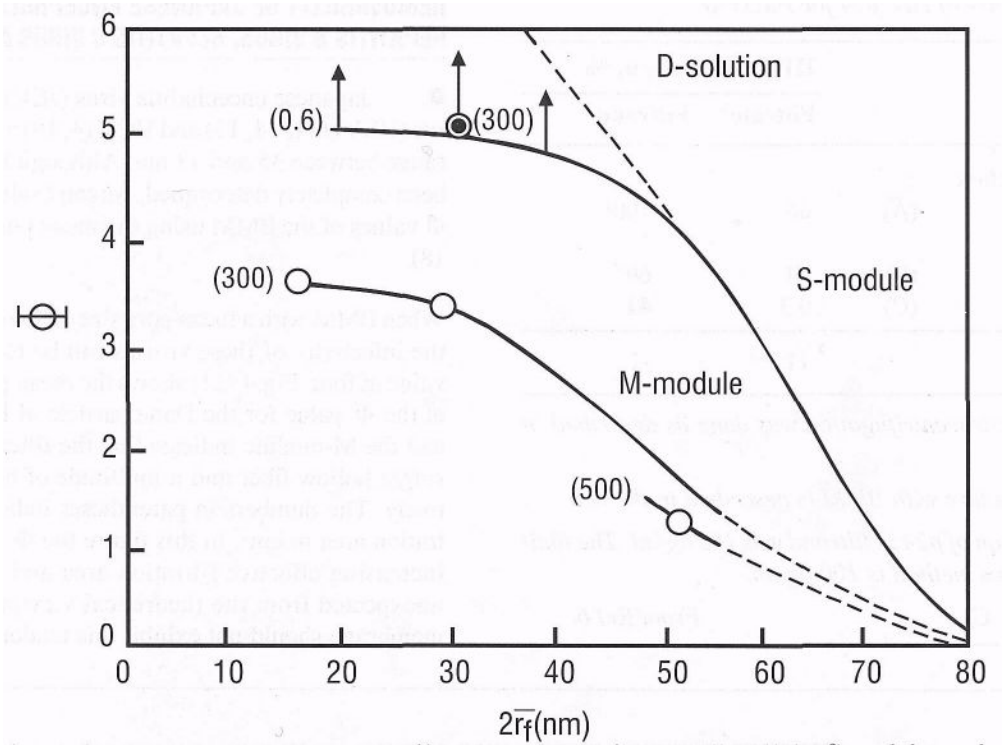

Figure 4. Virus logarithmic rejection coefficient <t> of hepatitis B virus for a small module (S-module) and a medium module (A>i-module). The numbers in parentheses indicate filtration area in cm^2. The broken line stands for the extrapolated value. 0 - 0 value for the M-module whose bubble point is more than 1 aim. © - 0 value for the M-module -whose bubble point more than 10 atrn. Arrows indicate that 0 value is more than that indicated by the arrow. From Ref 11.

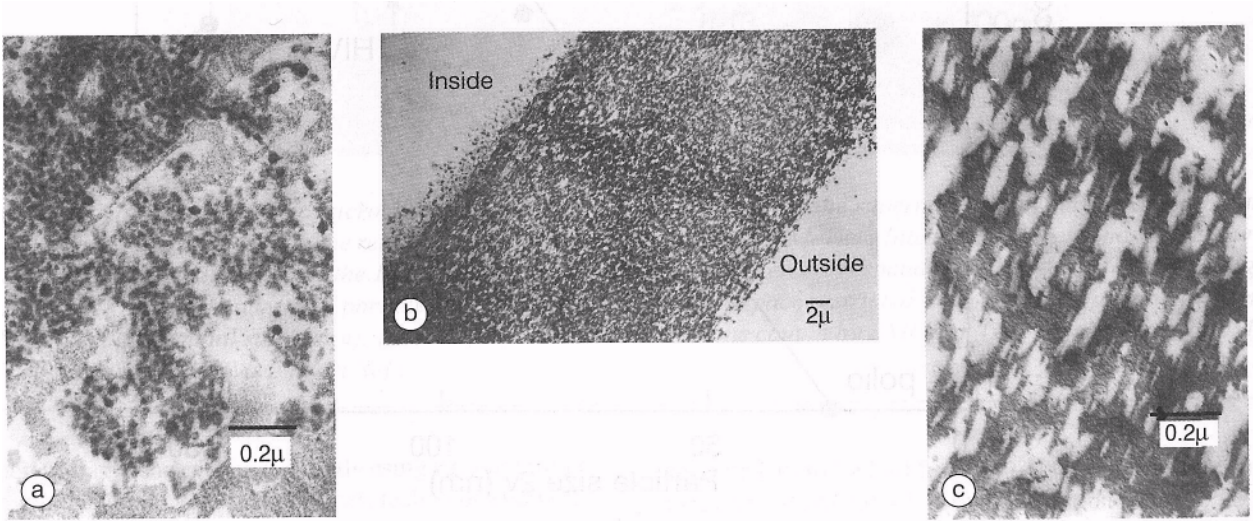

***Figure** 5. Electron micrographs of cross section ofBMMSO after filtration of Dane particles. The inner (a) and outer (c) parts of the membrane. The entire cross-sectional view of the membrane (b).*

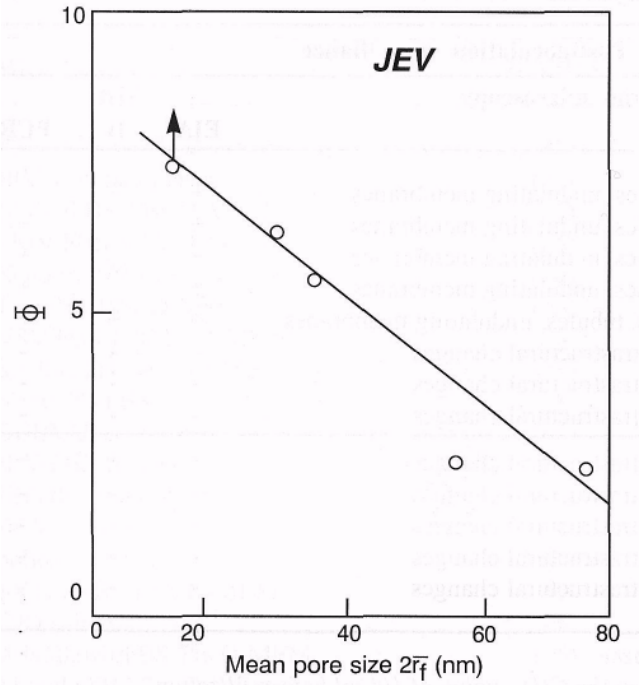

Figure 6. Dependence of 0 of Japanese encephalitis virus (JEV) on mean pore size ofBMM.

a constant value independent of the effective filtration area. This prerequisite can be achieved through the novel validation test method that we have proposed (20).

The electron microphotographs of Dane particles entrapped in a pore of the BMM are shown in Fig.5 (21). The Dane particles are packed like the eggs of a frog indicating that they have been caught through the plugging and trapping mechanism (21). The antigens of HBV such as HBs, HBe and HBc can pass easily through the BMM having a mean pore size larger than 35 nm.

The removability of JEV for the various BMM is summarized in Fig. 6. The empirical relation expressed by equation 2 was obtained between <*b* and $2r_f$ (in nm).

(2) $$\Phi = (215/2r_r) - 1.1$$

This equation is consistent with that derived from the theoretical approach (8). The JEV virion was employed as the standard virion to validate the BMM filter. When the filtration condition is kept constant, then the following empirical equation is derived. The reliability of this equation has been confirmed from the theoretical approach too.

(3) $$\Phi = Cl(2v/2r_f)d - C2$$

where Cl and C2 are constants independent of virion size 2v, mean pore size $2r_f$ and membrane thickness d. Equation 3 is applicable to most viruses and/or particles in an isolated monodispersed state (9). Fig.7 represents particle size dependence of O value for four kinds of BMM: BMM15 (whose mean pore size is 15 run), BMM35 (35 nm),BMM40 (40 nm) andBMM75 (75 nm) (22) The proteins of immunoglobulin G (IgG) can almost completely pass through BMM15, BMM35, and BMM40 indicating that most antibodies can pass through BMM under conditions excluding the contaminating viruses.

In the case of HC V, because of the lack of a susceptible cell line to propagate the virus, a polymerase chain reaction (PCR) as the assay method was employed (17). Although this method was very sensitive in terms of HCV detection, the existence of a part of the RNA of HCV does not necessarily mean the presence of an infectious virion. Next, an *in vivo* infection assay using chimpanzees in order to evaluate the concentration of HCV infectious particles was performend (17). Table 2 summarizes the results of the chimpanzee test for the HCV removal experiment, indicating that BMM35 can remove the infectious virions of HCV under the level of a 4 fold logarithmic reduction.

This level is consistent with that derived from the RNA removal data evaluated by the PCR method (16). Some pharmaceutical manufacturers have been employing BMM35 practically in their purification process based on their original data of HCV removal using BMM35 and their products have been approved by the Japanese government (23, 24).

Microorganisms other than viruses, such as mycoplasma (25), the agent causing Creutzfeldt-Jakob's disease (CJD) (26), bacteriophage (25) and bacteria, can also be removed using a series of BMM filters with various pore size. In the first case, the mycoplasma deforms into a slender shape under the shear stress caused by the fluid stream resulting in a smaller cross sectional diameter than that of the usual shape under a static state. The CJD agent has not been observed clearly, but is named prion protein. The removability of this agent by BMM35 indicates that the causative agent of CJD forms an aggregate that is more than 40 nm in size (23, 26). In the case of bacteriophage, removability is higher than expected from its size and shape because of the formation of aggregates under normal conditions. Table 3 summarizes the O values of various microorganisms for five BMM with a different mean pore size.

In the actual use of the BMM with a procured kidney to minimize HCV infection, not only the removability of HCV, but also the filtration capacity and filtration rate must be higher than the levels under actual operational conditions. When BMM35

***Table** 2. Results of chimpanzee test through injection offlltrand and filtrate*

HCV dose CID50	Filter pore/ steps	Animal	Postinoculation surveillance ALT ^	Histology	Electron microscopy	EIA	RIBI II	PCR
	none*	CH-420	+	Hepatitis	Tubules, undulating membranes	+	+	
	40 nm +	CH-422	+	Hepatitis	Tubules, undulating membranes	+		+
	50 nm	CH-424	+	Hepatitis	Tubules, undulating membranes	+		+
10^4	none*	CH-101	+	Hepatitis	Tubules, undulating membranes	+		+
	35 nm	CH-671	+	Hepatitis	Rings, tubules, undulating membranes	+	+	+
		CH-565	-	No lesions	No ultrastructural changes	-	-	-
	35 nm	CH-520	-	No lesions	No ultrastructural changes	-	-	-
	x 2	CH-533	-	No lesions	No ultrastructural changes	-	-	-
	none**	CH-418	+	Hepatitis	No ultrastructural changes	+	+	+
	35 nm	CH-546	-	No lesions	No ultrastructural changes	-	-	-
10^3		CH-569	-	No lesions	No ultrastructural changes	-	-	-
	35 nm	CH-587	-	No lesions	No ultrastructural changes	-	-	-
	x2	CH-569	-	No lesions	No ultrastructural changes	-	-	-

+ poisitive, - negative; injection volume was 1 ml for each chipmanzee.
**filtrand of the albumin solution containing hepatitis C virus having the CID value oflO^/ml before filtration.*
***filtrand with CID_X of 10^3/ml before filtration.* *From Rel18.*

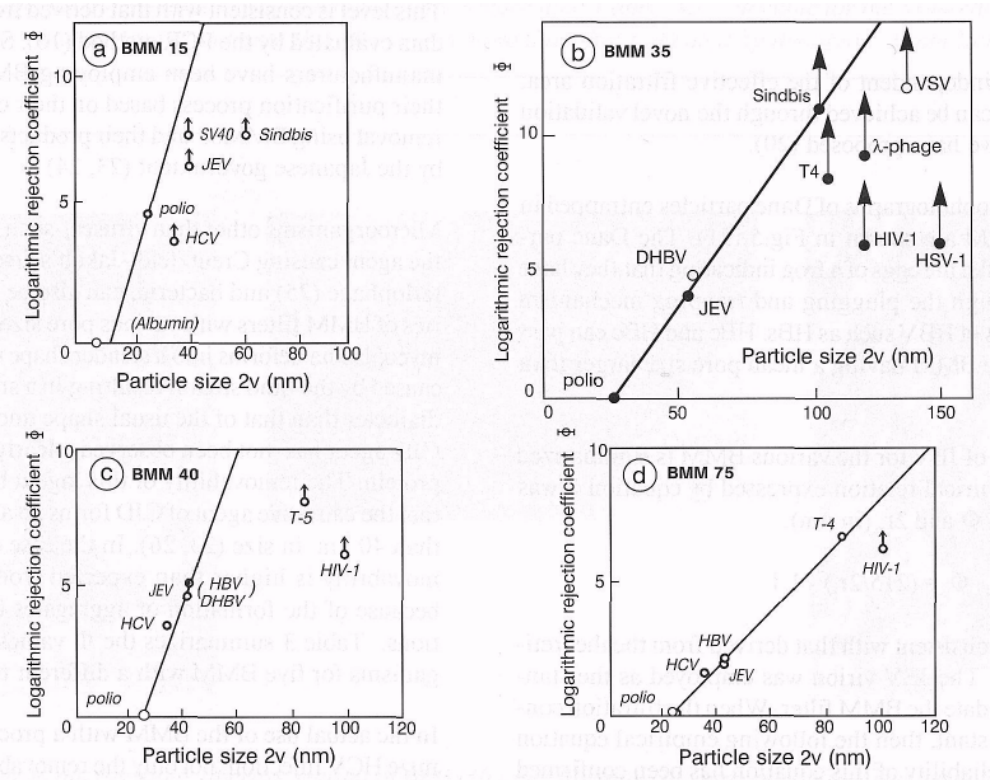

***Figure** 7. Particle size dependence of 0for various viruses for BMM15 (a), BMM35 (b), BMM40 (c) and BMM75 (d)*

***Table** 3. Logarithmic rejection coefficients of microbes for various BMM with different mean pore size*

Microbe/medium	BMM15	BMM35	BMM40	BMM50	BMM75
HSN-1/FBS 10% D-MEM	(>6)	>6	(>6)	(>6)	(>6)
HIV-1/human plasma	(>5)	>5	>5	>5	>5
HIV-1/FBS 10% D-MEM	(>6)	>6	>6	>6	>6
CMV/FBS 10% D-MEM	(>5)	>5	>5	>5	>5
Xenotrope/5% FBS	(>8)	>8	(>8)	(>8)	(>8)
T-4/FBS 10% D-MEM	(>8)	>8	>8	7.8	6.5
VSV/FBS 10% D-MEM	>8	>12	>12	>12	NT*
Sindbis/FBS 10 %D-MEM	(>8)	>8	>8	NT*	NT*
SV40/5% FBS	>8	6.5	(4.5)	(2.5)	(1.5)
DHBV/duck plasma	(>6)	6	5	NT*	NT*
JEV/FBS 10% D-MEM	>7	5.5	4.5	2.3	1.8
HBV/human plasma	(>6)	6	5	2.5	1.9
HCV/human plasma	>4	4	3,5	(2)	1.5
polio/FBS 10% D-MEM	5	0.2	(0)	(0)	(0)
ϕX174/FBS 10% D-MEM	>9	9	8	4	3
CJD/saline	>6	>6	4	3.5	2.2
A.laidlawii/FBS 7% D-MEM	(>5)	>5	>5	>5	>5
M.orale/FBS 7% D-MEM	(>5)	>5	>5	>5	>5
M.hyorhinis/FBS 7% D-MEM	(>5)	>5	>5	>5	>5

** NT - not tested, ( ) - estimated value.*

was placed in series with the preservation apparatus, high levels of HCV removal from the perfusion solution and an adequate speed of filtration were achieved (27).

As for the prion protein, the spread of BSE in cattle in Europe has posed a serious problem in the field of biotechnology. The BSE has potential for infection of humans since most of its biological characteristics are similar to those of CJD, which infects humans. Since bovine plasma has been an essential component of culture medium and may continue to play a vital role in biothechnology, validation of the purification process in the manufacture of a biodrug will be essential to protect from this infection (28). We have established a novel integrity test system for the validation membrane filter PLANOVA^{R} (20,29, 30). It has led to a drastic increase in reliability of the quantitative level of virus removability.

REMOVAL AND PERMEATION OF GENE DNA

• Government regulations demand the removal of DNA molecules from biodrugs. According to the recommendations of the Food and Drug Administration of the USA, the content of residual DNA in a biodrug should be less than 100 pg/dose (1-3). These recommendations are based on the strategy to increase the safety of the biodrugs. In contrast, good separation of DNA from the virus is utilized in the purification of DNA. Technology for the separation and purification of DNA may be applied in gene therapy in the future.

Fig. 8 shows the molecular weight dependence on permeability of DNA without the coexistence of proteins for the case of BMM35 and BMM15.

$$\Phi = C/C \quad (4)$$

where C_f and C_0 are the particle concentration in a filtrate and a feed solution, respectively. The permeability decreases linearly with increased in molecular weights (MW). The MW50 is defined as the MW at permeability of 50 %. MW values for BMM15 and BMM35 are $2xlO^{s}$ and $2xl0^{9}$, respectively (31). When DNA density in an aqueous solution $p_d$is assumed to be 1.7 g/ml, the corresponding volume Vd of the molecules with MW of 10^{s} and 10' are calculated to be $IxlQ^{-16}$ cm^5 and $1x10^{-15}$ cm^3, respectively, using the following equation;

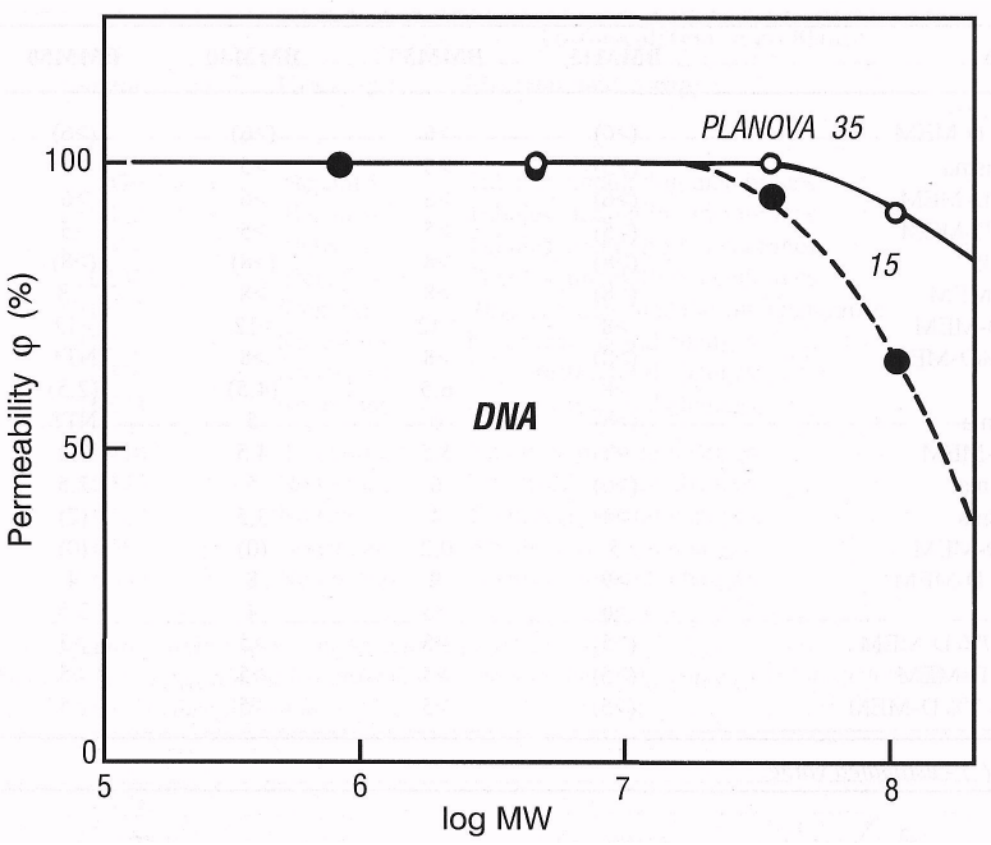

Figure 8. *Molecular weight (MW) dependence on permeability <pofDNAforPLANOVA33 and PLANOVA15. The concentration of the proteins coexisting in the solution can be neglected.*

(5) $Vd = MW/Na.p_d$

where Na is Avogadro's number. If the DNA molecule with the above Vd assumes a spherical shape, then the size of both molecules are approx. 60 nm and approx.130 nm for MW of 10^8 and 10^9, respectively. These sizes are far beyond the mean pore size of the BMM. Consequently, the DNA molecule may easily deform into a yarn-like shape when the shear stress acts on the molecule in the pore. This speculation is confirmed through electronmicroscopic observation of the BMM after filtration of a solution containing DNA molecules. Fig.9 shows a cross-sectional view of a BMM hollow fiber (31, 32). The yarn-like-shaped DNA molecules can be observed at the inside of the membrane wall. When the permeability of DNA molecules is controlled based on their deformability by shear stress generated in a pore, the DNA molecules can be purified and/or separated easily based on their MW. When protein molecules coexist, permeability of the DNA molecule diminishes drastically (33).

In the production of proteins by the use of biotechnology, DNA molecules change with the reaction time (34, 35). Consequently, with PLANOVA", the removability of DNA produced during the bioreaction changes with the reaction time. The recovery rate of proteins can be kept at a high level during the entire reaction time (35).

REMOVABILITY OF IMMUNE COMPLEXES AND PERMEABILITY OF PROTEINS INCLUDING MONOCLONAL ANTIBODY

• Rheumatic factors in human plasma can be removed through a BMM with a mean pore size of less than 30 nm (36), indicating that most immune complexes have a large particle size of more than 20 nm, which corresponds to the size of IgM.

Fig. 10 summarizes the dependence on the permeability of proteins in a solution having a concentration of less than 1% of their MW. When MW value increases the permeability of the protein decreases linearly as is the case of DNA molecules. The MW_{30} for BMM35 is $4x10^7$ and is about one fiftieth of that of

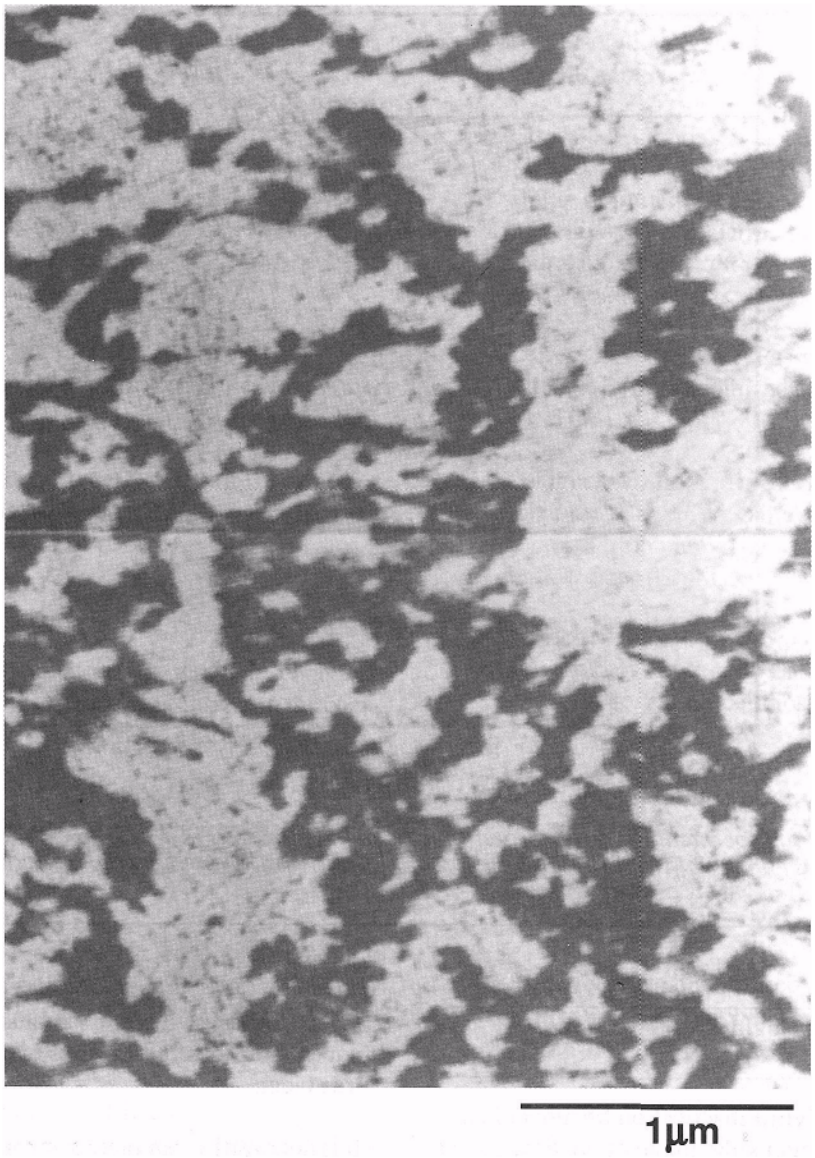

Figure 9. *Cross-sectional view of BMM after filtration of a solution containing DNA. The yarn-like shaped DNA molecules can be obsen'ed on the inside of the membrane wall. The DNA molecules are stretches within the BMM wall due to the shear stress originating from the filtrate flow in a pore.*

the DNA molecule. We can speculate from comparison with the case of DNA molecules that a series of globulins having an inherent molecular shape with a bulky configuration can not be deformed by shear stress into a yarn-like shape, a tendency that is the same as that of viruses (37).

The dependence of permeability of proteins on their MW for the BMM series with various mean pore sizes indicates that when a BMM with an appropriate mean pore size is employd can selectively be segregated harmful immune complexes without loss of valiable protein. Compared with plasma exchange for the removal of immune complexes from the human body, plasma perfusion with the BMM causes only minimal side effects due to the antigens transfused, such as viruses.

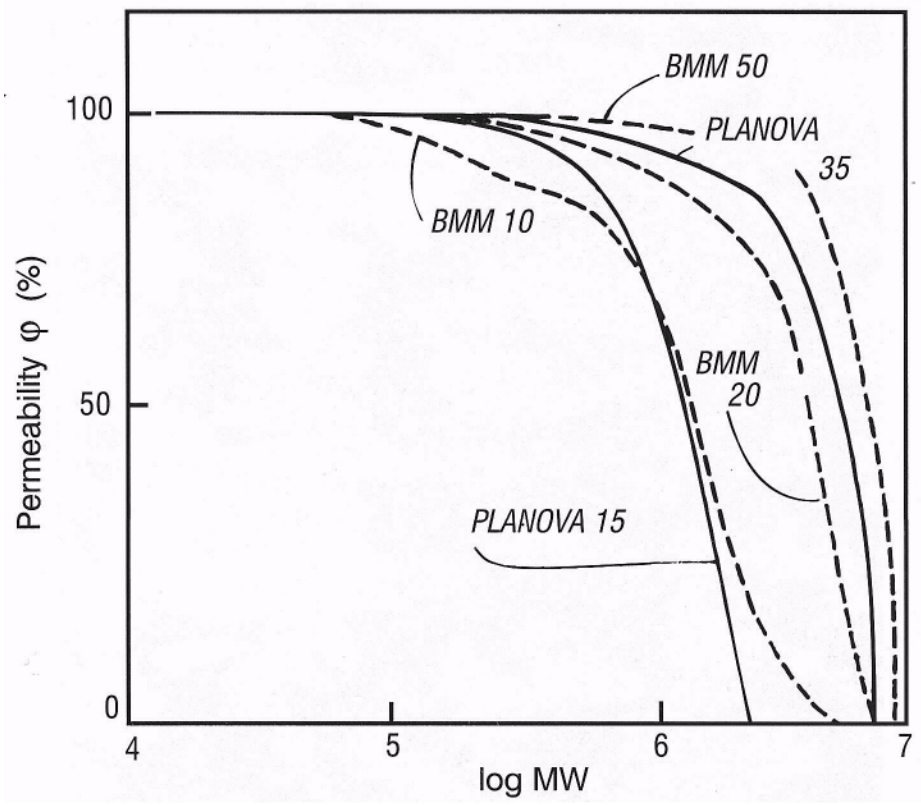

Figure 10. *Dependence of permeabilitv of a protein on its molecular weight (MW) for BMAI10, BMM20, BMM50, PLANOVA15, and PMNOl''A35. Broken line - BALM series, fall line - PI.ANOVA series.*

COMBINATION OF VIRUS REMOVAL USING BMM AND INACTIVATION

• The employment of virus inactivation treatment in addition to virus removal explains the range of applications of the virus removal filter to include use in protection from virus infections. Problems in the actual application of BMM arise when the target virus is small since the permeability and/or recovery rate of effective proteins for human health decreases with a decrease in the mean pore size of the BMM for virus removal. This problem can be overcome by taking aggregates of the viruses by the respective antibodies and/or by combining virus removal with virus inactivation treatment. The former solution seems to be possible in the case of parvo virus and of the causative agent of CJD *(26).* The latter solution can be carried out easily and is already utilized. The total degree of decrease in virus infectivityby the process of virus removal and virus inactivation is nearly equal to the summation of that of each treatment when virus removal is conducted after virus inactivation and more than the summation in the case of the reverse order of these treatments (38). The removal of components that obstruct the inactivation of viruses accelerates the treatment.

It is noteworthy that there is an optimum combination of virus removal and virus inactivation processes including their sequence. Examples include heat-treatment after filtration using BMM35 whose mean pore size is 35 nm (38) and ultraviolet treatment after filtration using BMM35 (39).

CONCLUSION

• A validation membrane such as the BMM for virus removal allows predictability of this process. Its mechanism is based on a sieving effect. Consequently, this membrane can remove not only the virus, but also particles such as immune complexes, low density lipoproteins and others generated in the body fluid. The content of particles in human plasma tends to increase with the age of the donor indicating that the plasma composition can be adjusted to that of children by filtration

using BMM. Although application to the manufacture of uncontaminated "pure" drags has been implemented, the actual clinical application of BMM requires further investigation.

The mechanism of particle removal (for example, the reduction of the population of virions in a body) is different from that after the injection of drags. Accordingly, we can also expect useful effects when particle removal and drag administration are combined. This is also the subject of a forthcoming study.

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REFERENCES

1. Center for biologies. Evaluation and research. Food and Drug Administration, USA. Points to consider in the characterization of cell lines used to produce biologicals. 1993; 1-40

2. CPMP Guidelines, Ad Hoq working party Biothechnology/Pharmacy. Note for guidance. Validation of virus removal and inactivation procedure, 1991

3. Hayakawa H. Evaluation of viral safety of biotechnology products derived from cell lines of human or animal origin. In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 1-8

4. Manabe S. Virus remodal and inactivation in process validation. In: Murakami et al, editors. *Animal Cell Technology. Basic and Applied Aspects.* 1992; 4: 15-30

5. Waterson AP, Wilkinson L. An introduction to the history of virology. Cambridge University Press, Cambridge, 1978; 29

6. Hamamoto Y, Harada S, Kobayashi S, Yamaguchi K, lijima H, Manabe S *et al.* A novel method for removal of human immunodeficiency virus: filtration with porous polymeric membrane. *Vox Sang* 1989; 56: 230-236

7. Manabe S, Tsurumi T, Ishikawa G, Satani M, Yamashiki T, Hamamoto Y *et al.* Mechanism of human immunodeficiency (HIV) removal by regenerated cellulose hollow fiber (BBM). *Membrane* 1989; 14: 77-83

8. Ishikawa G, Hirasaki T, Manabe S, Uematsu S, Yamamoto N. Novel determination method of size of virus in solution using cuprammonium regenerated cellulose membrane (BMM). *Membrane* 1991; 16: 101-111

9. Hirasaki T, Tsuboi T, Noda T, Uematsu S, Ishikawa G, Kono A *et al.* Removability of virus particles and permeability of protein from cell culture medium using cuprammonium regenerated cellulose hollow fiber (BMM). In: Murakami *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1992; 4: 49-55

10. Lefrere JJ, Vittecoq D. Passive immunotherapy in AIDS: a double blind randomized phase II study. *Vox Sang* 1994; 67: 234

11. Sekiguchi S, Ito K, Kodayashi M, Ikeda H, Tsurami T, Ishikawa G *et al.* Possibility of hepatitis B virus (HBV) removal from human plasma using regenerated cellulose hollow fiber (BMM). *Membrane* 1989; 14: 253-261

12. Mariko M, Ihikawa G, Yamaguchi K, Okada Y, Watanabe K, Sasaki-Iwaki *Yetal.* Concentration of live retrovirrus with a regenerated cellulose hollow fiber, BMM. *Arch Virol* 1994; 139: 87-96

13. Tsurumi T, Sato T, Osawa N, Hitaka H, Hirasaki T Yamaguchi K *et al.* Structure and filtration reformance of improved cuprammonium regenerated cellulose hollow fiber (improved BMM hollow fiber) for virus removal. *Polymer J* 1990; 22: 1085-1100

14. Ito K, Kobayashi M, Ikeda H, Sekiguchi S, lijima H, Tsurumi T *et al.* Possible removal of plasma virus by BMM porous hollow fiber. *Igakunoayumi* 1987; 142: 53-54

15. Sekiguchi S, Ito K, Kobayashi M, Ikeda H, Manabe S, Tsurami T *et al.* An attempt to prepare hepatitis B virus (HBV)-free plasma by ultrafiltration using micro-porous regenerated cellulose hollow fiber. *TransfSci* 1990; 11: 211-216

16. Yuasa T, Ishikawa G, Manabe S, Sekiguchi S, Takeuchi T, Miyamura T. The particle size hepatitis C virus estimated by filtration through microporous regenerated cellulose fiber. / *Gen Virol* 1991; 72: 2021 -2024

17. Yamamoto N, Ishikawa G, Hirasaki T, Manabe S, Yamashiki T, Yamaguchi K *et al.* Determination of size of HCV infectious particle and removal of the particle through ultrafiltration method using cuprammonium regenerated cellulose hollow fiber (BMM) [abstract]. 39th Ann Meet Soc Virol, Fukuaka, Japan, 1991

18. Muchmore E, Manabe S, Ischikawa G, Hirasaki T, Alter H, Shih J *et al.* Chimpanzee inoculation studies confirm removal of hepatitis C virus (HCV) infectivity by microporous membrane and estimate HCV particle size [abstract]. Int Symp Viral Hepatitis Liver Dis, Tokyo, 1993; 3196

19. Tateichi J, Kitamoto T, Ishikawa G, Manabe S. Removal of causative agent of Greutzfeldt-Jakob Disease (CJD) through membrane filtration method. *Membrane* 1993; 18: 357-362

20. Nakano H, Manabe S, Uematsu S, Sato T, Osawa N, Hirasaki T *et al.* Novel validation method of virus removability for biological cell culture products using polymeric membrane filters. In: Murakami *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1992; 4: 87-102

21. Yamaguchi K, Hamamoto Y, Manabe S, Yamamoto N. Microparticle removability of the regenerated cellulose hollow fiber (BMM). An electron microscopic evaluation. *J Electron Microsc* 1994; 40: 337-345

22. Sato T, Noda T, Uematsu S, Tsuboi T, Yamamoto N. Some characteristics novel BMM (BMM 15) for removal of polio virus from cell culture medium. In: Kaminogawa S *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1991; 5: 295-301

23. Tomokiyo K, Shigenobu A, Ogata Y, Tanigawa H, Funatsu A, Ohashi K *et al.* Studies on virus elimination and inactivation effects of highly purified F-VHI concentrate (EP-8). *Clin Rep* 1991; 25: 271-275

24. Tomokiyo K, Terano T, Shigenobu A, Tanigawa T, Funatsu A, Ohashi *Ketal*. Use of BMM membrane in the production of plasma derivatives [abstract]. *Virological Safety Aspects of Plasma Derivatives.* Int Ass Biol Standard, Cannes, France, 1992; 109

25. Manabe S, Umeda M, Kono A, Togo I, Fukada S, Yamaguchi K. Elimination of microorganisms from cell culture medium using regenerated cellulose hollow fiber (BMM). In: Sasaki R, Ikuka K, editors. *Animal Cell Culture and Production ofBiologicals.* Kluwer Academic Publishers, Netherlands, 1991; 87-94

26. Tateichi J, Kitamoto T, Ishikawa G, Manabe S, Yamaguchi K. Removal of the prion protein using validatable filter (BMMR). In: *Annimal Cell Technology, Basic and Applied Aspects.* 1995; 7: In press

27. Zucker K, Cirocco R, Roth D, Olson L, Burke GW, Nery J *et al.* Deplation of hepatitis C virus from procured kidneys using pulsatile perfusion preservation. *Transplantation* 1994; 57: 832-840

28. Colle JG. Stocktaking 1993. *Lancet* 1993; 342: 790-793

29. Sato T, Noda T, Manabe S, Tuboi T, Fujita S, Yamamoto N. Integrity test of virus removal membrane through gold particle method and liquid forward flow test method. In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 517-522

30. Noda T, Manabe S, Uragami T, Higuchi A, Sato T. Theoretical approach for integrity test of virus removal filter. In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 523-528

31. Hirasaki T, Yamaguchi K, Kono A, Noda T, Uematsu S, Tsuboi T *et al.* Removability and permeability of DNA in a solution by cuprammonium regenerated cellulose hollow fiber (BMMTM) for process validation of purification process of biodrug. In: Kaminogawa S *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1993; 5:273-279

32. Hirasaki T, Sato T, Tsuboi T, Nakano H, Noda T, Kono *Aetal*. Permeaton mechanism of DNA molecule in solution through cuprammonium regenerated cellulose hollow fiber (BMMTM). *JMembrSci* 1995; 106: 123-129

33. Mizusawa S, Sato T, Fujita S, Kobayashi R, Misu K, Honda R *et al.* Removal of residual DNA in biological products by cuprammonium regenerated cellulose hollow fiber (BMMTM). In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 537-541

34. Hirasaki T, Yamaguchi K, Kono A, Noda T, Sugahara T, Yamamoto N *et al.* Removability and permeability of DNA in protein solution using BMMTM. In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 543-547

35. Sugahara T, Noda T, Tsuboi T, Hirasaki T, Yamamoto N, Sakaeda S *et al.* Vims contaminant continuous culture of animal cells. In: Kaminogawa S *etal,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1993; 5: 233-237

36. Hayashida Y, Hamamoto Y, Manabe S, Kurimoto S, Yamamoto N. Removal of plasma immune complex in SLE patients through the microporous regenerated cellulose membrane hollow fibers. *Igakunoayumi* 1990; 152: 183-184

3 7. Hirasaki T, Ishikawa G, Nakano H, Manabe S, Yamamoto N. Mechanism of virus removal by regenerated cellulose hollow fiber (BMMTM). *Membrane* 1995; 19: 135-142

38. Tsuboi T, Hirasaki T, Noda T, Nakano H, Ishikawa, Uematsu S *et al.* Combination of virus removal and inactivation for the process validation of biodrugs. In: Kaminogawa S *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1993; 5: 289-294

39. Tsuboi T, Yamamoto N. Combination of virus removal and inactivation for optimization of recovery of protein. In: Kobayashi T *et al,* editors. *Animal Cell Technology. Basic and Applied Aspects.* 1994; 6: 9-15

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