再生セルロース中空糸 (BMM) を用いた 人血漿からの B 型肝炎ウイルス除去の可能性

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POSSIBILITY OF HEPATITIS B VIRUS (HBV) REMOVAL FROM HUMAN PLASMA USING REGENERATED CELLULOSE HOLLOW FIBER (BMM)

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We intended to show the applicability of the regenerated cellulose hollow fiber (BMM) to the preparation of plasma products free from hepatitis B virus (HBV). In order to evaluate the HBV removability of BMM and to exclude the contribution of the heterogenities of the hollow fibers which will affect the ability of BMM, two types of module (i.e. a S-module with its effective filtration area of 0.6 cm² and a M-module with 300 cm² or 500 cm²) were employed. The purified HBV particle were prepared from HBV positive plasma by rate zonal centrifugation method. The concentration of HBV particles in the samples were assayed by the spot hybridization method for HBV-DNA.

The virus logarithmic rejection coefficient Φ for HBV was approximately expressed by the following empirical and reduced equations,

$$\Phi_1(\text{exp}) = 7 / (2 \overline{r_f} - 15) \text{ for free HBV-DNA (empirical)}$$

$$\Phi_2 = 96 / (2 \overline{r_f} - 30) \text{ for HBV particles (reduced)}$$

where $2 \overline{r_f}$ was the mean pore diameter in nm unit obtained through the water filtration rate method.

When HBV positive human plasma was filtered by M-module, the observed Φ value of HBV-DNA was always between Φ_1 and Φ_2 because of the free HBV-DNA in plasma and heterogenities of the hollow fibers. The filtration rate was proportional to $(2 \overline{r_f})^2$ and was more than 3 l/m² · hr at the transmembrane pressure of 200 mmHg and the sieving coefficient of the total plasma protein was more than 90% when $2 \overline{r_f}$ was larger than 30 nm.

Key words : Regenerated cellulose hollow fiber (BMM), Hepatitis B virus, Virus-free,
Ultrafiltration, Plasma

The recent progress of screening hepatitis B virus (HBV) carriers in blood donor gives rise to the abrupt decrease in transfusion associated HBV infection. But there still remain some reported cases of the transfusion associated HBV infection¹⁾. This may be caused by the insufficient sensitivity of screening methods to exclude HBV infected blood donors. In order to increase the safety and quality of blood products, new approaches of virus removal or virus inactivation without denaturation of plasma proteins should be anticipated.

A membrane has been expected to be applied in getting virus free plasma products because of its moderate operation condition. Except for our preliminary experiments^{2~5)}, no attempt succeeded in this purpose. High performances by membrane are required for plasma fractionation. Firstly, the sieving coefficient (SC) of plasma proteins such as albumin and immunoglobulin G (IgG) should be larger than 90%. Secondly, the virus logarithmic rejection coefficient (Φ)*should be larger than 4.

Of HBV markers, the infective Dane particle is 42 nm in diameter and hepatitis B surface antigen (HBsAg) exists as tubular form with 22 nm in its cylindrical diameter or cubic particle having 22 nm in diameter. Hepatitis e antigen (HBeAg) is presumed to contain molecularly dispersed form and complex form with albumin or IgG in plasma⁷⁾ and its molecular weight (MW) is about 16 K. As for plasma proteins, albumin (MW=68 K) and IgG (MW=150-155 K) are 5~7 nm and 7~12 nm in diameter in aqueous solution, respectively⁸⁾. In order to get the high performance, we must clear up two problems, i. e. how to determine the HBV concentration and how to prepare the high performance membrane. It must be noted that the value of HBV concentration should reflect the amount of Dane particles since only Dane particle

is infectious among HBV markers. As for the membrane preparation, we must design the raw material which minimizes adsorption of proteins and is processed to have pore structure sharply enough to separate HBV from plasma proteins. Also the module constructed by the membrane should be homogeneous without any fault in pore structure.

This study is concerned with the applicability of the regenerated cellulose hollow fiber (BMM) with multi-layer structure⁹⁾ for the preparation of HBV-free plasma products. In order to reduce the possibility of the structural fault in the module, we will employ the single hollow fiber with the effective filtration area of 0.6 cm² (referred to as S-module) and also the module with the area of 300 cm² or 500 cm² (referred to as M-module). Four HBV preparations will be used in this study. They are (1) the partially purified Dane particles, (2) HBV-DNA solution free from Dane particles, (3) the normal plasma mixed with Dane particles and (4) the plasma positive for HBsAg and HBeAg, in order to get exact understanding of the removability of Dane particles by BMM.

EXPERIMENTAL

Sample Preparation : (a) BMM ; the hollow fibers were prepared from the cellulose cuprammonium solution through the microphase separation method under the severe control of the spinning condition¹⁰⁾. The mean pore size ($2\bar{r}_f$) was estimated from the filtration rate of water and ranged between 10 nm and 80 nm. (b) Module ; two types of module were employed ; S-module with 0.6 cm² in its effective filtration area and M-module with 300 cm² or 500 cm². Table 1 summarizes characteristics of BMM and module employed. (c) Partially purified Dane particles were obtained by the method described by Takahashi et al.¹¹⁾ with minor modification : The 20 l of HBsAg positive plasma was briefly centrifuged at 10200 g for 10min to clear out cell components and the su-

* $\Phi = \log_{10} N_0/N_f$ (1)⁶⁾
where N_0 and N_f are virus concentrations before and after filtrations, respectively.

Table 1 Characteristic values of BMM and its module

Sample code	Mean pore size $2\bar{r}_f$ (nm)	Porosity $Pr\rho$ (%)	Diameter		Wall thickness (μ m)
			inner (μ m)	outer (μ m)	
BMM 10	8-12	36 ± 5	250 ± 20	300 ± 20	25 ± 2
BMM 20	18-22	38 ± 5	250 ± 20	300 ± 20	25 ± 2
BMM 30	27-33	40 ± 5	250 ± 20	300 ± 20	25 ± 2
BMM 40	36-44	43 ± 6	250 ± 20	300 ± 20	25 ± 2
BMM 50	45-55	45 ± 6	250 ± 20	300 ± 20	25 ± 2
BMM 60	55-65	45 ± 7	250 ± 20	300 ± 20	25 ± 2
BMM 70	65-75	50 ± 7	250 ± 20	300 ± 20	25 ± 2

Module	Filtration area (cm ²)	Number of H. F*. s in a module	Length of H. F*. (mm)	Cut off bubble point (atm)	Housing dimension		Potting
					diameter (mm)	length (mm)	
S-module	0.6	1	80	9.0	1.5	9.5	polyurethane
M-module	300	500	80	1.0 or 10.0	21	94	polyurethane
M-module	500	800	80	1.0	21	94	

*H. F., Hollow fiber

pernatant was centrifuged at 33000g for 18hr at 4°C. For the enrichment of Dane particles, the pellets were subjected to floating type ultracentrifugation using a batchtype Hitachi RPZ 35-T rotor after reconstituted in plasma and the density adjustment to 1.30 g/ml. The fractions with the density of 1.15 to 1.25 g/ml were pooled. The pooled fractions were then subjected to rate zonal centrifugation to separate Dane particles from HB, Ag particles. Dane particles were localized in their action with sucrose concentration between 36% and 44%. They were concentrated and dialyzed with saline and about 100 ml of partially purified Dane particles was obtained. (f) Free HBV-DNA solution; the HBV-DNA extraction from HBsAg positive plasma was followed through Bemingen et al.¹²⁾ with minor modifications. Ethanol precipitation of HBV-DNA was dissolved with TE buffer (20 mM Tris-HCl, 5 mM EDTA in pH=7.5). The solution was heated at 70°C for 10min in order to denature the DNA before the BMM filtration.

Measurement; (a) Ultrafiltration; three types of filtration as shown in Figure 1 were carried out: Type (a) is dead end/constant transmembrane pressure type in which the constant pressure (ΔP) is applied with a manual pressure holder (left) or gravity (right). Type (b) is also dead end type and equipped with pressure pump which gives constant filtration rate. Type (c) is open feed type. Filtrate comes out while sample is recirculating through M-module under the constant pressure ΔP . The shear rate of the solution at the inner wall surface of BMM (γ) was calculated by putting the observed value of flow rate (Q) of the filtrand, the inner diameter of the hollow fiber (D), and the total numbers of the hollow fiber constituting the module (n) into eq (2) derived for the laminar flow fluid.

$$\gamma = 2(2Q-J) / \left\{ \pi n(D/2)^3 \right\} \quad (2)$$

The filtrations were carried out through the dead end filtration under the constant transmembrane pressure ΔP (type (a) filtration in Fig. 1) when there is no description for the filtration type.

(b) Porè characteristics; the mean pore size $2\bar{r}_f$

Fig. 1. Schematic representation of three types of filtration procedure.

(a) ; dead end/constant transmembrane pressure in the case of S-module (left hand side) and M-module (right hand side), (b) ; dead end/constant filtration rate, (c) ; open feed/constant pressure under the constant shear rate, 1 ; sample, 2 ; pressure driver i. e., pump in the cases of type (b) and type (c), and manual pressure holder in type (a), 3 ; pressure gauge, 4 ; BMM module, 5 ; filtrate reservoir, (ΔP ; transmembrane pressure)

was determined by eq (3) putting the observed values of J (ml/min in unit) for pure water, ΔP (mmHg), wall thickness of the hollow fiber d (μ m), effective filtration area A (cm²), apparent porosity $Pr\rho$ (-), and viscosity of water η (centipoise),

$$2\bar{r}_f = 2.0 \times (J \cdot d \cdot \eta / \Delta P \cdot A \cdot Pr\rho)^{1/2} \quad (3)$$

Here, the $Pr\rho$ was evaluated under the assumption that the density of regenerated cellulose solid was 1.5 g/ml.

(c) HBV assay ; the amounts of HBsAg and HBeAg were assayed by reversed passive hemagglutination (R-PHA)⁽¹³⁾. In this method, the presence of HBsAg or HBeAg induces agglutination of red cells which are coated with respective antibodies. The concentration of HBV-DNA was determined by the spot hybridization method⁽¹²⁾ with ³²P-labelled cloned HBV-DNA as probe. After

phenol-ethanol treatment, sample DNA was spotted on a nitrocellulose filter and specific binding with the radiolabelled HBV-DNA probe was monitored by autoradiography.

(d) Protein assay ; Total protein was determined from the absorbance at 590 nm a dye-binding method using Tonein-TP (Ohtuka assay, Japan). Human serum albumin was as protein standard.

RESULTS AND DISCUSSION

1. Ultrafiltration of purified Dane particles with S-module

Dane particles were concentrated from HBV carrier plasma and partially purified by rate zonal centrifugation as described in Experimental. The fractions corresponding to 38~44% sucrose density contained Dane particles with small amount of HBsAg and no detectable HBeAg. The value of X_1 , defined as weight ratio of the free DNA dissociated from Dane particle to total amount of DNA was obtained through the procedure shown in Appendix I and was 0.0004.

Type (a) filtration with S-module was carried out with the partially purified Dane particles (D-solution). As shown in Fig. 2a the virus logarithmic rejection coefficient of HBV-DNA (Φ) and sieving coefficient of HBsAg (SC) was dependent on the mean pore size ($2\bar{r}_f$). The Φ value for Dane particle (Φ_2) and free HBV-DNA (Φ_1) were calculated from the observed Φ values in Fig. 2a and X_1 according to the procedure shown in Appendix I. The calculated values of Φ_1 and Φ_2 are shown in Fig. 2b with observed values of free HBV-DNA. The calculated Φ_1 is larger than the observed, suggesting that the size of free HBV-DNA depends on its source or preparing conditions. There was an inverse relationship between the Φ_2 value for Dane particles and $2\bar{r}_f$. When $2\bar{r}_f$ is smaller than 51 nm, Φ_2 is larger than 4. This level of virus rejection (i. e. $\Phi=4$) is nearly equivalent to the inactivation level of HBV by heat-treatment employed in the plasma fractio-

Fig. 2. Dependences of HBV logarithmic rejection coefficients (Φ) and sieving coefficient (SC) on the mean pore size ($2\bar{r}_f$) of the BMM module.

Type (a) filtration was carried out with the S-module under the constant pressure of 200 mmHg. (a) Φ values of HBV-DNA (closed circle) and SC of HBsAg (open circle). Arrows indicate the values are over (or under) the detectable level in the indicated direction. (b) Φ values of free HBV-DNA (Φ_1) and Dane particles (Φ_2), full lines indicate the reduced value obtained through the procedure shown in Appendix I. Closed circle represents the observed Φ value of free HBV-DNA.

nation products¹⁴⁾. When $2\bar{r}_f$ is smaller than 46 nm, Φ_2 becomes larger than 6. If this level of Φ_2 is achieved by the filtration of HBV positive plasma, Dane particles may not be detectable in the filtrate even if few amounts of HBsAg are detected.

The HBsAg is detected at least in Dane particles and surface antigen particles (tubular and cubic forms). Molecularly dispersed form of HBsAg has not been recognized yet. The diameters of the surface antigen particles are smaller than 42 nm.

Consequently, Φ for Dane particles should be always larger than $\log(SC)$ of HBsAg. The results in Fig. 2 support this prediction. The dependences of each Φ on $2\bar{r}_f$ are described by the following empirical and reduced equations (the value of $2\bar{r}_f$ is adopted in nm unit). That is, the Φ_1 value obtained for the free HBV-DNA solution was expressed as $\Phi_1(\text{exp}) = 7/(2\bar{r}_f - 15)$, and the reduced values of Φ_1 and Φ_2 in Fig. 2 (b) were expressed as

$$\left. \begin{aligned} \Phi_1 &= 35/(2\bar{r}_f - 15) \text{ for free HBV-DNA (reduced)} \\ \Phi_2 &= 96/(2\bar{r}_f - 30) \text{ for HBV-DNA in Dane particle (reduced)} \end{aligned} \right\} (4)$$

The asymptotic value of Φ with a decrease in $2\bar{r}_f$ into 20 nm was 5.2 and was nearly equal to the sum of $-\log X_1$ and Φ_1 . This indicates that when $2\bar{r}_f$ approaches to 20 nm, the HBV-DNA in the filtrate is only from soluble component and not from Dane particles.

In Figure 3, the correlation of filtration rate with the mean pore size was shown. When D-solution was applied on S-module at $\Delta P = 200$ mmHg and filtration volume of 2 l/m^2 , the dependence of the filtration rates of HBV and pure water on the mean pore size can be expressed as following equations as a function of $(2\bar{r}_f)^2$,

$$J = 0.062 \times (2\bar{r}_f)^2 \text{ for pure water} \quad (5)$$

$$J = 0.014 \times (2\bar{r}_f)^2 \text{ for Dane particle-rich solution (D-solution)} \quad (6)$$

The difference between eqs (5) and (6) can be explained due to the difference in viscosity of the samples and not due to the Dane particles in the solution.

2. Ultrafiltration of Dane particles with M-module

In order to apply the BMM to the process of plasma fractionation or the bed side transfusion, the module with larger effective filtration area is necessary. The newly developed M-module consists of 500 of hollow fibers with effective filtration area of 300 or 500 cm^2 . However, the Φ value of the

Appendix I Estimation of purity of Dane particles from apparent logarithmic rejection coefficient Φ_a of HBV-DNA

Since there is no direct method to assay the infectivity of HBV in vitro, the concentration of HBV-DNA is often utilized as the index to represent the amounts of HBV (Dane) particles. However, it reflects not only virus associated DNA but also free DNA dissociated from Dane particles.

Now, we define, X_1 as the weight ratio of free HBV-DNA to total HBV-DNA in order to express the purity of Dane particles in a given preparation. The X_1 value of 1.0, for example, indicates that the 100 % of the measured DNA is free DNA dissociated from HBV particles. The X_1 value can be estimated by three consecutive filtration steps of D-solution through S-module on the assumption that the free HBV-DNA and Dane particles go through the BMM module independent of each other and the rejection coefficients of the two DNAs are determined by the membrane characteristics.

An apparent logarithmic rejection coefficient Φ_a for HBV-DNA is defined as eq. (A-1) ;

$$\Phi_a = \log_{10}(C_o/C_f) \quad (\text{A-1})$$

where C_o and C_f are the concentration of HBV-DNA of original (prefiltration) solution and filtrate, respectively. After the first step filtration, C_f is given theoretically by eq. (A-2) ;

$$C_f = X_1 C_o 10^{-\Phi_1} + (1-X_1) C_o 10^{-\Phi_2} \quad (\text{A-2})$$

$$\text{or } \Phi_a = \log_{10}(C_o/C_f) = -\log X_1 10^{-\Phi_1} + (1-X_1) 10^{-\Phi_2}$$

where Φ_1 and Φ_2 are the rejection coefficients of free DNA and Dane particles, respectively. The Φ values for HBV-DNA at the second (Φ_{II}) and the third (Φ_{III}) step filtrations are obtained by the following eqs. (A-3) and (A-4) ;

$$\Phi_{II} = -\log X_1 10^{-2\Phi_1} + (1-X_1) 10^{-2\Phi_2} \quad (\text{A-3})$$

$$\Phi_{III} = -\log X_1 10^{-3\Phi_1} + (1-X_1) 10^{-3\Phi_2} \quad (\text{A-4})$$

The three consecutive filtrations of D-solution will give Φ_I , Φ_{II} and Φ_{III} . Thus, the X_1 , Φ_1 and Φ_2 values can be calculated by putting the observed Φ values into eqs. (A-2), (A-3) and (A-4).

Fig. 3. Dependence of filtration rate (J) on the mean pore size.
○ ; pure water, ● ; D-solution, △ $P=200$ mmHg, filtration volume = 2 l/m^2 .

module as a whole is mainly influenced by the defective hollow fiber which has the minimum value of Φ as is shown theoretically in Appendix II. The-

efore, it is most important to exclude such defective fibers from the module in order to attain the level achieved by S-module. The detailed technique about the segregation of defective fibers will be given in different paper¹⁵⁾.

Figure 4 shows comparison between Φ values of S-module and M-module in the filtration of D-solution. The modules with pinholes were excluded in advance by checking its bubble point higher than 1 atm or 10 atm in ethyl alcohol. The filtration volume was fixed at 2 l/m^2 in all cases. The Φ value decreases with an increase in the effective filtration area, which is proportional to the total numbers of hollow fibers, indicating the increase in the possibility of straying of defective hollow fibers in the module. When the improved hollow fibers with the bubble point of 10 atm are employed

Appendix II Relationships between virus logarithmic rejection coefficient of a module (Φ_m) and that of hollow fibers (Φ) constituting the module

The value of Φ_m is given as a function of Φ in principle although it is difficult to conceive the detailed form of the function at a first glance. In order to understand the relationship between Φ_m and Φ quantitatively, an approximation equation relating both values will be derived below.

Define the distribution function of Φ , $N(\Phi)$, as the possibility that the value of Φ of a hollow fiber is between Φ and $\Phi + d\Phi$ is expressed by $N(\Phi) d\Phi$. So to speak $N(\Phi)$ is the frequency distribution function of Φ . Then, the equation (A-5) is derived from the definition of $N(\Phi)$.

$$\int_0^\infty N(\Phi) d\Phi = 1.0 \quad (\text{A-5})$$

Assume that the filtration rates of hollow fibers constituting the module in question are same to each other under the common filtration condition then the concentration of virus in the filtrate N_f is given by

$$N_f = N_o \int_0^\infty 10^{-\Phi} N(\Phi) d\Phi \quad (\text{A-6})$$

where N_o is the virus concentration of the original solution before the filtration (prefiltration). The equation (A-6) is derived from the definition of Φ_m as follow,

$$\Phi_m = -\log \left(\int_0^\infty 10^{-\Phi} N(\Phi) d\Phi \right) \quad (\text{A-7})$$

We employ the three typical functions of $N(\Phi)$ shown in Figure A-1. Substitution of these functions into eq. (A-7) gives us the value of Φ_m . Figure A-2 shows the value of Φ_m as a function of $\Delta\Phi$. Here, $\Delta\Phi$ is the difference between the maximum value of Φ , Φ_{max} , and the minimum one, Φ_{min} , i. e., $\Delta\Phi = \Phi_{max} - \Phi_{min}$. The contribution of Φ_{min} is larger than that of the nominal mean value of Φ , $\bar{\Phi}$, defined as $\int_0^\infty \Phi N(\Phi) d\Phi$.

The values of Φ_m for the above three cases of $N(\Phi)$ can be approximately expressed by the following equation obtained using the results shown in Fig. A-2.

$$\Phi_m = 0.25 \bar{\Phi} + 0.75 \Phi_{min} \quad (\text{A-8})$$

It is clear from eq. (A-8) that the effective rejection of virus by the module is realized by increasing Φ_{min} under the same value of $\bar{\Phi}$.

Fig. A-1. Schematic representation of frequency distribution function of Φ , $N(\Phi)$, employed numerical calculation.

Φ_{min} ; minimum value of Φ among those of hollow fibers constituting M-module, Φ_{max} ; maximum value of Φ , $\Delta\Phi$; distribution width of Φ , i. e., $\Phi_{max} - \Phi_{min}$.

Fig. A-2. Φ value of M-module whose distribution of Φ is given in Fig. A-1.

Φ_m ; Φ value of M-module. The symbols of a, b and c stand for the modules whose distribution of Φ are given by a, b and c in Fig. A-1, respectively.

Fig. 4. HBV logarithmic rejection coefficients of S-module and M-module. The numbers in the parentheses indicate the effective filtration area in cm^2 . Broken line stands for the extrapolated value. $\circ$; Φ values for M-module whose bubble point is 1 atm or higher. $\odot$; Φ values for M-module whose bubble point is 10 atm or higher. Arrows indicate value is over detectable level.

for the M-module, the Φ value approaches to that of S-module as is shown in Fig. 4. The differences between Φ values of S- and M-module for D-solution can be minimized by increasing the cut-off level of the bubble point to exclude defective fibers from M-module.

When we employed the Dane particles mixed with HBs negative fresh-frozen plasma (referred to as P-solution), the Φ value of S-module decreased about three degree in Φ value (data is not shown here). This decrease was due to the increase in X_1 value of P-solution from 0.0004 of D-solution to 0.05. As is the case of D-solution the differences between Φ values of S- and M-modules for P-solution also became smaller by using hollow fibers with higher bubble point. The Φ_1 (exp) of free HBV-DNA in the P-solution could be approximated by the following empirical equation (7).

$$\Phi_1(\text{exp}) = 18/(2\bar{r}_f - 15) \quad (7)$$

3. Filtration of HBV positive plasma with the BMM module

Figure 5 shows the dependence of the Φ values

Fig. 5. Logarithmic rejection coefficients of HBV in human plasma. $\circ$; S-module, $\bullet$; M-module, Δ ; Φ value of S-module for free HBV-DNA. P-plasma; Dane particles were mixed with human plasma whose X_1 value was 0.02 and applied on the BMM module. Arrows indicate the value is over the detectable level.

on $2\bar{r}_f$ for HBV positive plasma (referred to as P-plasma). The Φ values for P-plasma were considerably lower than those for D-solution. This is probably due to the X_1 value which is 0.02 for P-plasma. This value may vary depending not only by the melting condition of fresh-frozen P-plasma but also the physical condition of the original donor of the plasma. Using eqs (5) and (7) and the X_1 value, the Φ_2 value of HBV for P-plasma in M-module is expressed by the following equation.

$$\Phi_2 = 50/(2\bar{r}_f - 30) \quad (8)$$

Figure 6 summarizes the Φ values for P-plasma in M-module under three types of filtration procedure with the filtration volume of 1 l/m^2 . The transmembrane pressure was 200 mmHg in the case of types (a) and (c) and varied from 0 to 380 mmHg in the case of Type (b). Type (c) filtration gives the highest value when the same mean pore size and same ΔP are employed. This may be caused by the fractionation mechanism related to fluid dynamics for dispersed particles as proposed before¹⁶⁾. The difference in Φ values among the three types of filtration procedure tends to disappear with a decrease in $2\bar{r}_f$.

The SC value of protein for M-module as a whole can be expressed as an average SC value of

Fig. 6. Differences in HBV logarithmic rejection coefficients by filtration types. P-plasma was applied on type (a) filtration ($\circ$), type (b) filtration ($\bullet$), and type (c) filtration ($\triangle$). The filtration volume is 1 l/m^2 . Arrows indicate the value is over detectable level.

Fig. 7. Sieving coefficient of plasma total protein and its dependence on the mean pore size. Type (a) filtration was carried out with fresh frozen plasma and M-module. Filtration volume is 1 l/m^2 under the constant pressure of 200 mmHg.

individual hollow fiber. In the case of plasma proteins, the contribution of the heterogeneity of hollow fibers to SC value is not as large as the case of the Φ value of HBV. Therefore, we can employ M-module for plasma proteins to analyze the filtration characteristics of BMM.

Figure 7 shows the dependence of the SC value of plasma protein on $2 \bar{r}_f$, when the fresh frozen plasma is applied to M-module at the filtration volume of 1 l/m^2 and ΔP of 200 mmHg. When $2 \bar{r}_f$ is larger than 20 nm, most of proteins apparently flow through BMM. However, it should be noted that the SC value of each plasma component is largely dependent on the size of the component. For example, at the $2 \bar{r}_f$ of 30 nm, the SC value of albumin is more than 0.9 whereas that of factor VIII is 0.25. More than 90% of plasma proteins are albumin and γ -globulin which are smaller sized molecules. The SC value also depends on ΔP , filtration type and filtration volume.

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(受付 1989. 4. 27)