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The Size of Serum Hepatitis Virus.* (19809)

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The agent of serum hepatitis (SH) is known to pass through various "bacteria-retaining" filters (Seitz EK(1,2) and Berkefeld N(3)), but the size of the virus, like that of infectious hepatitis (IH), has so far remained undetermined. The present report is concerned with experiments designed to determine the size range for SH virus by means of ultrafiltration of known icterogenic serum through a series of gradocol membranes† of various average pore diameters (A.P.D.)(4).

Materials and methods. Acute phase serum (strain J. W.) was obtained from an American soldier in Germany on the second day of jaundice (30 September 1949). It was kept in a CO_2 dry ice box for 42 days and then at −20°C until the filtrations were carried out in July and November of 1951.‡ A portion of this same lot of serum had previously (1950) produced clinical and laboratory evidence of hepatitis in 3 volunteers, each of whom received 0.5 cc subcutaneously and 0.1 cc intracutaneously(5). The incubation period at that time ranged from 53 to 87 days. In the first series of filtrations whole undiluted serum was used. After clarification through a Seitz EK pad a portion of the serum was passed through a 300 mμ, A.P.D. gradocol membrane under 12 lb positive pressure (nitrogen gas) at 38-39°F. This was then used as a "master" filtrate, portions of which were filtered through membranes of 200 mμ, and 94 mμ A.P.D. under similar conditions. In the second series of filtrations 10% serum in normal saline was used, the procedure being the same as above except for the addition of a 52 mμ membrane. This dilution factor was tried in order to facilitate filtration which is a most time-consuming procedure when whole undiluted serum is used. All filtrates were kept frozen until the time of inoculation. Twenty-nine volunteers, quartered in the same institution,§ participated in the present study. All were healthy males (aged 21 to 30) who gave no history of previous jaundice of any type. All had normal liver function tests (*i.e.*, direct and total serum bilirubin, cephalin fluocculation, thymol turbidity and flocculation, and alkaline phosphatase) at the time of inoculation.

Exp. I. Seventeen volunteers were divided into 4 groups, each receiving a single filtrate

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† Obtained from the Inoculation Department, St. Mary's Hospital, London.

‡ Filtrations were carried out with the aid of Dr. Joel Warren in the Virus and Rickettsial Disease Laboratory, Army Medical Service Graduate School, Washington, D.C.

§ Federal Correctional Institution, Danbury, Conn. Acknowledgment is made to Mr. Allen Shank and Mr. L. C. Schilder, Wardens, and Dr. Leon Witkin, Chief Medical Officer, for their permission to allow these men to volunteer and for their generous assistance and cooperation during the observation period.

TABLE I.
Summary of Results with Various Filtrates.

		Whole serum	10% serum
Unfiltered		+ + +*	Not used
Filtrate	Seitz EK	+ + — —	" "
	300 mμ	+ + — —	" "
	200	± — — — —	+ — — —
	94	± — — —	+ — — —
	52	Not used	+ — — —

Each sign (+, ± or —) indicates one volunteer inoculated.

+ = definite laboratory and/or clinical evidence of hepatitis.

— = no evidence of hepatitis.

± = borderline elevations of serum bilirubin on one occasion only.

* Three tests representing an earlier exp.(5).

of whole serum (Seitz and membrane filters of: 300 mμ, 200 mμ, or 94 mμ). The inoculum consisted of 0.4 cc subcutaneously and 0.1 cc intracutaneously. *Exp. II.* Twelve volunteers were divided into 3 groups, each receiving a single filtrate of 10% serum in normal saline (200 mμ, 94 mμ, or 52 mμ). The total inoculum used was 5 cc given in multiple subcutaneous injections. During the post-inoculation period in both experiments all volunteers were interviewed and examined at approximately weekly intervals. Liver function tests were repeated 3 and 6 weeks after inoculation and at one to 2 week intervals thereafter until the termination of the experiment at the end of 4 months. Individually numbered syringes and needles were used in order to avoid any possibility of cross inoculation. The volunteers were not isolated except during the period of acute illness. No cases of hepatitis had been observed in the institution for one year prior to the present study and none was observed outside the experimental groups during the period of observation.

Results. The rates of occurrence of hepatitis among all of the inoculated volunteers are listed in Table I.

In the first experiment 2 of the 4 individuals receiving the Seitz filtrate and 2 of the 4 receiving the 300 mμ filtrate developed definite clinical and/or laboratory evidence of hepatitis. The degree of illness varied from extremely mild to moderately severe, with peak serum bilirubin levels ranging from 2.06 to 7.92 mg %. The average incubation period was 76.5 days, ranging from 73 to 87 days, and the average duration of illness was approximately 35 days. One member of each of the other groups (200 mμ and 94 mμ) showed borderline elevations of serum bilirubin levels at the same time that the above cases first became apparent. These "borderline" values were 2 to 3 times the levels previously observed in the same individuals, but they fell rapidly to normal and there were no other confirmatory laboratory and no clinical findings.

In the second experiment definite clinical and laboratory evidence of hepatitis developed in one member of each of the 3 groups (200 mμ, 94 mμ, and 52 mμ) of 4 inoculated volunteers. Each of these men became markedly icteric (peak serum bilirubin levels from 13.6 to 23.1 mg %) and the clinical course was much more severe than in Exp. I. Because of the marked nausea and vomiting and the difficulties involved in providing adequate parenteral nutrition, these men were all treated with cortisone (100 mg intramuscularly for 5 days, followed by the oral preparation in gradually decreasing dosage during the next 20 days). Coincident with this therapy, there was a prompt and dramatic subsidence of symptoms within the first 24 hours. Serum bilirubin levels decreased fairly rapidly during the first few days and continued a steady downward trend in 2 of the patients as the cortisone dosage was reduced. In the third patient, however, a sudden relapse occurred with the decrease in dosage, followed by prompt improvement when the dosage was increased. No relapse was noted during a period of 2 months after cortisone was discontinued in these 3 patients. The incubation period in this group ranged from 62 to 70 days, averaging 65 days. The duration of illness in each case was approximately 50 days.

A summary of the clinical and laboratory findings of both experiments, together with the results previously obtained by Evans(5) using the same (J.W.) serum unfiltered, is given in Table II.

Discussion. In the first experiment the results were equivocal with a questionable endpoint reached with the 300 mμ filtrate. It was felt, however, that whole serum might have produced sufficient plugging of the smal-

TABLE II.
Clinical and Laboratory Findings in 32 Volunteers Inoculated with SH Virus (J.W. Strain).

	Inoculum	No. with hepatitis / No. in group	Men who devel. abnormal liver function tests	Day of onset of symptoms	Day of onset of icterus	Liver exam.		Serum bilirubin* (highest level)		Avg peak serum bilirubin for group
						Enlargement	Tenderness	Direct, mg %	Total, mg %	
Evans (5)	Unfiltered whole J.W. serum (.5 cc subcut. .1 cc intracut.)	3 / 3	FT	53	53	no	yes	1.15	2.96	6.96
			BS	65	68	no	yes	4.90	9.96	
			CH	75	87	yes	yes	3.29	7.57	
Exp. I	Filtrates of whole J.W. serum (.4 cc subcut. .1 cc intracut.)									
	Seitz EK	2 / 4	JT	0	0	?	?	.89	2.06	5.12
			DS	75	80	yes	yes	3.53	5.98	
	300 mμ	2 / 4	WC	85	93	yes	yes	4.41	7.92	
			HL	82	95	yes	yes	2.53	4.52	
	200 mμ	0 / 5	TK†	0	0	no	no	.15	1.08	
	94 mμ	0 / 4	JB†	0	0	no	no	.24	1.38	
Exp. II	Filtrates of 10% J.W. serum (5 cc subcut.)									
	200 mμ	1 / 4	PM	60	62	yes	yes	11.60	23.1	17.27
	94 mμ	1 / 4	WM	75	77	yes	yes	7.42	15.1	
	52 mμ	1 / 4	RN	68	72	yes	yes	7.16	13.6	

* Upper limits of normal in this laboratory: .20 mg % direct; 1.30 mg % total.
† These results are included only because a "suggestive" rise in serum bilirubin levels occurred at the same time that abnormal levels were noted in other volunteers in the same experiment.

ler A.P.D. membranes to prevent passage of an infective dose of virus. That this was probably true was borne out by the second experiment using 10% serum filtrates which yielded evidence that the virus passed through the smallest (52 mμ) A.P.D. membrane used.‖

‖ Recent experiments(9) in this laboratory using filtrates of Coxsackie virus (size 15 to 23 mμ, titer $10^{-7.5}$) suspended in varying concentrations of horse serum indicate that extraneous protein interferes with passage of the virus, but that if the virus concentration is sufficiently high, there will be no effect on the filtration end point. Whole serum could not be filtered through the smaller A.P.D. membranes satisfactorily. The following table summarizes the results:

Filtrate, mμ	Negative logarithm of virus in Whole horse serum	10% horse serum	1% horse serum
140	5.1	>6.8	>6.8
87	5.5	6.5	>6.8
53	*	2.5	6
41	*	0†	0†

* Serum not filterable.
† 0 indicates max conc. of virus tested (.5% suspension of infected tissue) failed to infect any of 24 infant mice.

According to Elford's formula(6) the size of the virus would be 26 mμ or less. Since no end-point was reached the size range cannot be further limited.

It has been shown that the pores of collodion membranes of the type used are not uniform in size or configuration, that there is a wide variation in the pores of a single membrane(7), and that there may be variations in results obtained using membranes of the same production lot(8). Therefore, the results of a single experiment are open to considerable question and the value of 26 mμ or less is given with these facts in mind.

Another aspect of the results of these experiments should be noted, although it is again realized that the numbers involved in the tests are small and of questionable significance. When 10% serum filtrates were used instead of whole serum, the three illnesses produced were apparently more severe, of longer duration, and the incubation periods were relatively shorter. Since there is no way of knowing the variations in susceptibility of the indi-

viduals involved, no definite conclusions can be drawn from these observations and any explanation falls into the realm of speculation. It is suggestive, however, of a change in the infectivity of the virus in the process of dilution, perhaps concerned with a virus-antibody relationship.

Cortisone therapy appeared to have a definite and immediate beneficial effect in the three patients who were treated, but any conclusions concerning its value from this study would again be questionable since there was no adequate control group and the number of cases was small.

Summary. 1. Known icterogenic (SH) serum (J.W. strain), undiluted and diluted 10% in normal saline, was filtered through gradocol membranes of various average pore diameters down to 52 mμ, and tested in human volunteers. 2. Evidence has been presented that the strain of serum hepatitis virus used is small enough to pass through a 52 mμ A.P.D. membrane, thus indicating a size of 26 mμ or less. The limitations of the application of this method for determining the size of this virus are mentioned.

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In Vitro Conversion of Thiocyanate to Cyanide in the Presence of Erythrocytes.* (19810)

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The *in vivo* conversion of thiocyanate to cyanide has been reported recently by Goldstein and Rieders(1) in contrast to previous studies in which this reaction could not be demonstrated(2,3). Because thiocyanate has been widely used in the management of patients with hypertensive vascular disease, further study of this problem was undertaken in the course of investigation of thiocyanate-induced diuresis in man(4).

Methods. Cyanide was determined by the method of Gettler and Goldbaum(5). The material to be tested is acidified, heated to 90°C and aerated; the eluted hydrogen cyanide is carried to filter paper impregnated with ferrous sulfate, producing the Prussian blue reaction. The method is reported to be highly specific and sensitive to 0.0001 mg. For the purposes of the present study no effort was made to obtain sensitivity greater than 0.0005 mg. Standards were set up in whole blood and serum containing 0.0005, 0.001, 0.002, 0.003, 0.004, and 0.005 mg of cyanide;† the colored papers were preserved for comparison with the unknowns. The specificity of the method appeared to be adequate as judged by a limited number of observations. Repeated analyses of water, serum, plasma, and boiled whole blood to which varying amounts of thiocyanate had been added yielded no detectable cyanide. The development of the character-

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† The terms "cyanide" and "thiocyanate" refer to the anion only.