STUDIES ON THE NATURE OF THE AGENT TRANSMITTING LEUCOSIS OF FOWLS*

II. Filtration of Leucemic Plasma

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Recent studies have indicated that the mode of action of leucemic plasma is different from that of leucemic cells. Although inoculum containing leucemic cells (whole blood and cell suspensions) also contains some of the filterable transmitting agent, the leucemic cells are themselves capable of multiplication in susceptible hosts, like cells of transplantable tumors (1). On the other hand, leucemic plasma transmits leucosis only because it contains the agent which causes neoplastic transformation of some of the bone marrow elements of susceptible hosts. This agent is present in the plasma in very high concentration (2). Its properties in general, and particularly its filterability, are best studied with this material, which can easily be obtained free from cells and cellular debris.

Filtration through Silicious Filters

Previous work has already shown that the agent transmitting leucosis may pass Berkefeld filters (3–6). The difficulties of demonstrating filterability were assumed, as with some viruses causing infectious diseases, to be in part due to the association of the agent with particulate matter (*cf.* 7). In support of this view the observations of Pentimalli (8), may be quoted, who found that the virus of Rous sarcoma is easily adsorbed on susceptible cells. Rous and Murphy (9) assume that the agents transmitting tumors are of relatively large

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size as compared with other filterable causes of disease. They suggest two possibilities to explain the narrow range within which the agents of Rous tumors are filterable: first that they are formed bodies; second that if unformed, they are associated with substances that clog the pores of the filter. Occasional failures suggested to Gye and Andrews (10) that filterability is not an essential property of the Rous tumors. The evidence presented here on the filterability of the agent points to an explanation somewhat different from those suggested.

It seemed desirable to obtain accurate information as to the quality of the silicious filters that pass or retain the transmitting agent, and to prove that the coarser filters (N and V) retain all cells. That cells of the Rous tumors may pass coarse Berkefeld filters is maintained by several investigators (11).

Technique.—The filters used were new, of the smallest size manufactured (about 2.5 x 1.5 cm.), and were tested before sterilization. There are two measurements commonly applied to obtain data suitable for an estimation of the size of the pores of a filter (*cf.* 12). One of these, the "bubbling pressure," was determined as specified by the manufacturer. The second, the rapidity of the flow of water through the filter, was measured by us arbitrarily at a negative pressure of 40 cm. Hg after the flow of water had become uniform. With new filters of the same make the two measurements yielded parallel findings. Tests of each filter proved particularly desirable with the so called N and V types since they did not show constantly the characteristic differences indicated by their designations. W filters on the other hand were uniformly finer than either V or N filters.

The material filtered was plasma to which from 20 to 40 per cent heparin solution (1:1000) was added to prevent clotting. The plasma was further diluted by the addition of broth containing microorganisms, in most instances *Vibrio percolans* (13) and *B. prodigiosus*. The final dilution of the plasma used for filtration was from 1:2 to 1:3. In one instance only was it diluted considerably, as suggested by Ellermann. This material (Passage VIII C) was obtained by adding 1 cc. whole blood to 200 cc. isotonic salt solution and spinning the diluted blood. Since this experiment was unsuccessful whereas concentrated or only slightly diluted plasma after filtration produced leucosis, high dilutions were not used in subsequent tests.

The filtration was undertaken in most instances at about 400 mm. Hg below the atmospheric pressure. The negative pressure was somewhat greater in the first four tests (Tables I and II). The material to be filtered was chilled before filtration, as was the filter itself in several instances. The filters were readily permeable to the plasma, with the exception of two W filters (Passages XII F and XII H), through which the flow rapidly decreased and filtration was interrupted at the end of 10 to 12 minutes.

From 0.002 to 0.01 cc. of the filtrate was examined in the counting chamber in several experiments, and was invariably found to be free from cells (Table II).

Agar and broth cultures of filtered and unfiltered plasma were kept at 37.5°C. for from 1 to 2 days and subsequently at room temperature for from 2 to 4 days. The cultures of the material to be filtered showed in all instances heavy growth but the filtrates were sterile with two exceptions. *Vibrio percolans* passed one Mandler filter having a bubbling pressure of 320 mm. Hg (Passage XII B) and a Berkefeld N filter with a bubbling pressure of 400 mm. Hg (Passage XII F), but these filters retained *B. prodigiosus*. The failure of coarser filters to retain *Vibrio percolans* was to be expected from the studies of Mudd (13).

Fowls lost through intercurrent disease within the estimated period of incubation have not been included in Table I among the number of fowls injected. The figures given for the periods of incubation and duration of illness are based on examinations of the blood and are therefore approximate but not accurate.

The results of fifteen filtration tests, summarized in Table I, indicate that the success of filtration is less dependent on the porosity of the silicious filter than on such factors as the presence in the plasma of contaminating particles, and substances with affinity for silicious filters.

The agent present in the plasma transmitting leucosis seems to pass fine silicious filters readily if the plasma is free from small particles or substances that obstruct the pores. In two experiments direct filtration through a W filter (Passages XII F and XII H) led within a few minutes to almost complete obstruction and the filtrates were inactive. Filtration through a W filter when preceded by filtration through an N filter (Passage XI G) proceeded rapidly and the filtrate caused leucosis in two of four fowls injected.

The addition of heparin preserves the fluidity of the plasma and therefore facilitates filtration. In the filtration test just described 40 per cent heparin solution (1:1000) was added to the plasma. In the other tests collected in Table I, the plasma contained between 15 and 40 per cent heparin solution. Moreover the precautions usually taken for rapid preparation and handling of plasma in making tissue cultures were adopted to delay clotting.

Repeated centrifugalization at high speed, omitted in the first filtration tests for fear that it might lead to an inactivation of the transmitting agent, seems likewise advisable. Recent experiments have shown that the distribution of the agent in the plasma is not considerably affected by spinning for 2 hours at 3000 R.P.M. In a very

TABLE I

The Results of Filtration of Leucemic Plasma through Silicious Filters

Passage	Filtered plasma						Unfiltered plasma					Suspension of cells or whole blood				
	Type of filter	Amount injected	No. of fowls injected	Successful inoculation			Amount injected	No. of fowls injected	Successful inoculation			Amount injected	No. of fowls injected	Successful inoculation		
				No. of fowls	Period of incubation	Duration of illness			No. of fowls	Period of incubation	Duration of illness			No. of fowls	Period of incubation	Duration of illness
		cc.			*days*	*days*	*cc.*			*days*	*days*	*cc.*			*days*	*days*
VI C	V	1 to 8	7*	3	102, 139, 139	19, 21, 43						0.05	3	1	57	10
												1.5	2**	0		
VII C	V	0.8	9	2	59, 95	7, 63	0.5	4	3	59, 110, 117	10, 112, 33	0.5	4	1	75	13
							0.01	4	0			0.01	4	2	32, 95	7, 44
							0.0002	4	0			0.0002	4	0		
VIII C	V	0.05	5	0								1.0	6	1	17	7
		0.025	5	0								0.01	5	3	32, 49, 80	1, 7, 20
		0.01	5	0								0.0001	6	0		
VIII E	V	0.5 to 1	8	2*	17, 31	20, 27	0.5	2	1	15	1	0.5	5	4	9, 10, 12, 59	3, 3, 11, 13
							0.005	4	1	27	12	0.005	3	2	16, 52	3, 4
												0.00005	2	0		
X B	N	0.5 to 1	7	5	29, 32, 52	1, 1, 2	0.2	3	2	30, 45	6, 53	0.2	4	2	28, 29	3, 1
					52, 59	39, 41	0.001	4	2	45, 56	1, 3	0.001	4	2	68, 22	1, 10
							0.000005	4	1	56	35	0.000005	4	0		

XI B	N	1	6	2	40, 47	1, 8	1	3	1	45	5	0.5	6	6	15, 17, 18	5, 7, 9
	V	1	3*	0											45, 55, 70	18, 43
XI G	N + W	2 to 4	4*	2	37, 44	1, 2	1	3	2	23, 46	9, 22					
XII B	6 lb.	1.5	6	3	42, 47, 49	12, 14, 20	1	2	1	28	3	1.0	3	2	19, 19	4, 8
	9 lb.	1.5	5	3	21, 28, 31	6, 9, 13	0.01	2	1	98	13	0.01	3	3	27, 27, 32	13, 15, 23
												0.001	1	0		
XII C	8 lb.	0.6 to 3	3**	2	61, 101	1, 1	0.5	2	1	21	2	0.1	3	0		
												0.001	2	2	37, 98	7, 136
												0.00001	3	0		
XII F	N	0.3 to 1	5	1	42	39										
	W	0.3 to 1	3	0												
XII H	N	0.3 to 3	3*	1	100	4										
	W	0.4 to 1	2	0			1	3	1	53	1					

* One fowl of each of these groups had mild transient anemia with exception of a fowl in Passage XII H, which had severe anemia The causation of these anemias by the transmitting agent seemed uncertain and therefore they were grouped as negatives.

** One fowl in each of the two groups indicated had lymphoid leucosis.

successful filtration test, namely XII B, the plasma when inspected in the counting chamber was entirely free from cells before filtration.

TABLE II

Data on the Filtration Tests Given in Table I

Passage	Type of filter	Bubbling pressure	Flow of water per minute	Duration of filtration	Flow of material filtered	Bacteriological examination of the filtrate
		mm. Hg	*cc.*	*min.*		
VI C	Berkefeld V	—	—	30	Very slow	Sterile
VII C	Berkefeld V	—	—	5	Rapid	Sterile
VIII C	Berkefeld V	—	—	15	Fast	Sterile
VIII E	Berkefeld V	—	—	3	Rapid	Sterile
X B	Berkefeld N	400	—	3	Rapid	Sterile
XI B	Berkefeld N	480	—	4	Rapid	Sterile*
	Berkefeld V	400	—	—	Rapid	Sterile*
XI G	Berkefeld N followed by W	440	52	2	Rapid	—
		770	14	10	Fast	Sterile
XII B	Mandler 6 lb.	320	38	5	Rapid	Slight growth of *V. percolans* but not of *B. prodigiosus**
	Mandler 9 lb.	560	18	3	Rapid	Sterile
XII C	Mandler 8 lb.	430	30	5	Rapid	Sterile*
XII F	Berkefeld N	400	38	7	Fast	Slight growth of *V. percolans* but not of *B. prodigiosus**
	Berkefeld W	760	16	12	Slow, stopped	Sterile*
XII H	Berkefeld N	—	—	—	Rapid	Sterile
	Berkefeld W	—	—	10	Slow, stopped	Sterile

* These filtrates were examined in the counting chamber and were found to be free from cells.

Estimation of the Amount of Transmitting Agent Absorbed by Silicious Filters

A direct determination of the concentration of the agent in the plasma before and after filtration has not been made. Such a determination would require a considerable number of fowls since the figures obtained are significant only if the titration is complete. The percentage of successful inoculations with plasma, as shown in the preceding paper (2), is independent of the infecting dose within the very wide limits of about 10^{-2} to 10^{-6}.

Some intimation of the amount of agent present in the filtrate may be obtained by a comparison of the length of the incubation periods with filtered and with unfiltered plasma (Table III). It has been shown (2) that the incubation period is prolonged when the amount of plasma containing the transmitting agent is decreased. In estimating the effect of filtration upon the incubation period the amounts injected, as shown in Table III, must be taken into consideration. It will then be seen that in three of the nine filtrations the filtrate caused leucosis within about the same period of time as the unfiltered plasma, suggesting that approximately the same amount of the agent was contained in both. In the other six filtration experiments, however, the incubation period was slightly to moderately prolonged, suggesting a slight to moderate decrease in concentration of the agent as a result of filtration (2).

Discussion.—The experiments reported here leave no doubt that the agent transmitting leucosis is filterable through silicious filters. It seems essential that preceding the filtration, the solutions containing the agent be freed as completely as possible from particulate matter. Since the agent is present in high concentration in the plasma, the conditions for obtaining it free from cells and cellular débris are more favorable than with the agents of filterable tumors. Passage through fine filters may be facilitated by preceding filtration through a coarse filter. Similar results were obtained by Krueger and Schultz (14), who found that the virus of poliomyelitis was completely retained by a 2 per cent collodion membrane, but that when filtered first through a 0.5 per cent collodion membrane, it then passed the 2 per cent collodion filters. The mechanism underlying the success of filtrations

through fine filters after preceding filtrations through coarser filters is not clear *a priori*. Removal of particles larger than the agent is one possibility but removal of surface-active substances having an

TABLE III

Relation of the Period of Incubation of Leucosis Caused by Inoculations with Unfiltered Plasma and with Plasma Passed through Silicious Filters

Passage	Filtered plasma			Unfiltered plasma			Estimated effect of filtration on the period of incubation
	Amount injected	Period of incubation	Duration of illness	Amount injected	Period of incubation	Duration of illness	
	cc.	*days*	*days*	*cc.*	*days*	*days*	
VII C	0.8	77	35	0.5	95	52	No effect
VIII E	0.75	24	24	0.5	15	1	
				0.005	27	12	Moderate delay
X B	0.75	45	17	0.2	38	30	
				0.001	52	2	Moderate delay
				0.000005	56	35	
XI B	1	44	5	1	45	5	No effect
XI G	2	37	2	1	35	16	Slight delay
	4	44	1				
XII B (6 lb.)	1.5	46	15	1	28	3	Moderate delay
XII B (9 lb.)	1.5	27	9	0.01	98	13	No effect
XII C	0.6	61	1	0.5	21	2	Much delay
	2.5	101	1				
XII H	2	100	4	1	53	1	Much delay

If more than one fowl, inoculated with the same dose, developed leucosis average figures were given. With regard to inaccuracies in determining the period of incubation and the duration of illness, see notes to Table IV of the preceding paper.

affinity for silicious earth may likewise play some rôle. The results with filtration of the agent of leucosis suggest that under favorable conditions active agents of tumors may pass the finest silicious filters.

The view that successful inoculation with filtrates of tumor is due to a few cells that contaminate the filtrate would seem as unfounded as the view that cells may pass silicious filters under ordinary conditions of filtration. The filtrate and in some instances even the plasma used for filtration were free from cells when viewed in the counting chamber. In a few tests, the filtrate was centrifugalized at high speed and the sediment was inspected in the counting chamber but was found to be free from cells. Leucemic plasma, when incubated with embryonic extract at 37.5°C., showed no growth. In one experiment a very coarse (not bacteria-tight) silicious filter, having bubbling pressure of 20 cm. Hg. was used to test filterability of leucocytes in leucemic plasma but none passed through. The passage of phagocytes through living membranes with pores much less than their average size is beyond dispute. This however does not seem to take place under ordinary conditions of filtration through Berkefeld filters. Cooling the filter and material to be filtered may have inhibited active motion of leucocytes.

According to the estimations of Bechhold (15*c*) the maximum size of pores of W filters is from about 1.5 to 3.5μ and a microbe passes pores of silicious filters if it is from eight to fifteen times smaller than the pores of the filter. On the basis of these figures it may be assumed that the passage of a microbe through a W filter indicates that the microbe is smaller than 0.1 to 0.44μ.

Filtration of Leucemic Plasma through Collodion Membranes of Graded Porosity

Considerable knowledge has been gained recently concerning the technique of ultrafiltration and its application to studies of filterable microbes (*cf.* Bechhold (15), Elford (16), Krueger and Ritter (17)). Ultrafiltration may serve either to determine the size of viruses or to free them from extraneous matter, but thus far it has not been possible to obtain them in pure form, nor are the values given for their size beyond dispute (*cf.* 15). Valuable information can however be obtained by filtering a variety of biologically active substances through collodion membranes of graded prorosity under comparable conditions as has been done by Zinsser and Tang (18). The present study was undertaken to compare the filterability of the agent transmitting

leucosis with that of other particulate matter of submicroscopic size.

The passage of organic particles through collodion membranes under conditions determined by us are summarized in Table IV, and tests on the filtration of the agent transmitting leucosis in Table V.

In a series of *preliminary tests* the relation of pressure to the flow of water and to the passage of colloidal substances (Table IV) was determined. Such tests showed the maximum pressure that the various types of filters would resist; *e.g.*, Prussian blue was retained through 1.5 per cent collodion membranes when filtered

TABLE IV

Filtration of Colloidal Substances and Microorganisms through Collodion Membranes

Material filtered	Approximate diameter	Collodion membrane			
		0.5%	1.5%	3%	4.5%
	mμ				
B. prodigiosus	Above 800	Passed*	Retained	Retained	
Prussian blue	400	Passed	Retained	Retained	—
Virus of bovine pleuro-pneumonia	250	Passed*	Retained	Retained	—
Ferric oxide	100	Passed	Passed	Passed*	Retained
Arsenic trisulfid	90	Passed	Passed	Retained	Retained
Collargol	30	Passed	Passed	Retained	Retained
Bacteriophage	25	Passed	Passed	Passed*	Retained
Litmus	2	Passed	Passed	Passed	Passed
The agent transmitting leucosis		Passed	Passed	Passed*	—

* Concentration much decreased.

at a pressure of 4, 8, and 16 cm. Hg but it passed this filter in about one-eighth of the original concentration when the pressure was raised to 32 cm. Hg. On subsequent lowering of the pressure to 8 cm. Prussian blue continued to pass through the filter in about one-tenth of the original concentration indicating that the membrane had been altered. The filtrations described here were carried out at a pressure that according to preliminary tests would not injure the collodion membrane.

In all tests the leucemic plasma was obtained by spinning heparinized blood. The blood cells were suspended in Locke solution, shaken, recentrifugalized, and the supernatant liquid was added to the plasma. The final dilution of the plasma was from 1:2 to 1:3. The filtration of the agent transmitting leucosis through collodion membranes was carried out in the ice box.

TABLE V

Filtration of Leucemic Plasma through Collodion Filters

No. of passage	Material injected	Amount injected	No. of fowls injected	Successful inoculations		
				No. of successful inoculations	Period of incubation	Duration of sickness
		cc.			*days*	*days*
XVIII B	Plasma, unfiltered	0.5	4	1	76	15
	Same, passed through 0.5% collodion membrane	0.5	3	1	6	56
XVII D	Plasma, passed through Berkefeld N filter	0.4	3	1	16	19
	Same, after additional passage through 0.5% and 1.5% collodion membrane	1.5	4	1	25	5
XVII E	Plasma, passed through Berkefeld N filter	0.5	2	0	—	—
	Same, after additional passage through 0.5% collodion membrane	1.25	2	1	23	10
	Same, after additional passage through 1.5% collodion membrane	2.5	2	2	24, 26	19, 86
XIX D	Plasma, passed through Berkefeld V filter	0.5	3	1	24	10
	Same, after additional passage through 0.5% and 1.5% collodion membrane	1.0	3	2	34, 45	7, 9
	Same, after additional passage through 3% collodion membrane	1.5	3	0	—	—
XX D	Plasma, passed through Berkefeld V filter	1.0	3	1	25	5
	Same, after additional passage through 1.5% collodion membrane	1.0	3	2	31, 51	4
	Same, after additional passage through 3% collodion membrane	1.0	3	1	41	11
Total...	Unfiltered		4	1	76	15
	Passed through Berkefeld filter		11	3	16 to 25	5 to 19
	Passed through 0.5% collodion membrane		5	2	23 to 61	10 to 56
	Passed through 1.5% collodion membrane		12	7	24 to 45	4 to 86
	Passed through 3% collodion membrane		6	1	41	11

The 0.5 per cent *filters* used for preliminary filtration were prepared by us; the denser filters were purchased from Schleicher and Schüll. The filters were placed in a Seitz filtration apparatus supported by meshed wire and several layers of ordinary filter papers. The filters were washed with Locke solution immediately before filtration. The amounts injected are given in terms of the original volume of plasma.

A *bacteriophage* active against *B. coli* was obtained through the courtesy of Dr. F. B. Lynch. The broth culture, after lysis, was centrifugalized, the supernatant fluid passed through a coarse silicious filter and subsequently through a series of collodion filters (Table IV). The size of the bacteriophage is given according to the estimate of Burnet (19).

A culture of the virus of *bovine pleuropneumonia* was obtained through the courtesy of Dr. W. J. Elford. In one experiment the serum broth cultures were filtered directly through collodion membranes. In another test the filtration was made as described for bacteriophage.

Broth cultures of *B. prodigiosus* were diluted with about ten times the volume of Locke solution and passed directly through a 0.5 per cent collodion membrane; part of this filtrate was then filtered through a 1.5 per cent membrane.

The *colloidal substances* were filtered directly through the collodion membranes indicated in Table IV; a preceding filtration through coarse membranes did not improve their filterability through finer membranes.

In Table IV "retained" indicates that the substance filtered could not be demonstrated in the filtrate. However inspection of the lower surface of the membrane, which apparently just retained colored colloidal substances, often showed a discoloration indicating that the material has passed in minute amounts.

Passage XVIII B.—The plasma passed through the 0.5 per cent membrane in quantities of 5 drops per minute at a negative pressure of 12 cm. Hg. One of the fowls injected with unfiltered plasma and given in Table V as negative died of intercurrent disease 33 days after injection. The two fowls injected with filtered plasma and recorded as negatives had transient anemia.

Passage XVII D.—The plasma was not perfectly clear before filtration and its passage through the Berkefeld filter was very slow. This filter had a bubbling pressure of 40 cm. Hg and the flow of water per minute was 40 cc., at a negative pressure of 40 cm. Hg.

Passage XVII E.—The Berkefeld N filter used for preliminary filtration had a bubbling pressure of 41 cm. Hg and the flow of water was 48 cc. per minute. The filtration proceeded rapidly. The final filtration through 1.5 per cent membrane was very slow, about 15 cc. liquid passed the filter in $1\frac{3}{4}$ hours.

Passage XIX D.—The coarse silicious filter had a bubbling pressure of 25 cm. Hg and it permitted 53 cc. water to pass per minute. The filtration proceeded rapidly through the silicious filter, but it was slow through the 0.5 and 1.5 per cent membrane and the 3 per cent membrane was almost impermeable after 10 cc. liquid passed through it in the course of 50 minutes.

Passage XX D.—Preceding filtration the plasma was thoroughly cleared by repeated spinning. The coarse silicious filter used in this test had a bubbling pressure of 22 cm. Hg and the flow of water was 84 cc. per minute. The filtration lasted for 1½ hours and the passage of 10 cc. of liquid through a 3 per cent membrane required 1¼ hours.

Table V shows that the agent transmitting leucosis readily passed 1.5 per cent collodion membranes. Indeed, successive filtrations of plasma through a coarse Berkefeld filter, 0.5 and 1.5 per cent collodion membranes resulted not in a decreased but in an increased activity of the transmitting agent as indicated by the following percentage of successful inoculations: unfiltered plasma 25 per cent (see also Table III of the preceding paper), plasma after filtration through coarse silicious filter 27 per cent, 0.5 per cent collodion membrane filtrate 40 per cent, 1.5 per cent filtrate 58 per cent. These tests permit the conclusion that the agent readily passes 1.5 per cent collodion membranes and suggest the presence of inhibitory substances in leucemic plasma (*cf.* 20, 21). Only one of the six fowls injected with plasma passed through 3 per cent membranes, developed leucosis. This may be interpreted by assuming that the majority of the pores of 3 per cent membranes retain the agent.

DISCUSSION

It is frequently stated that viruses pass collodion filters that retain or barely permit the passage of proteins; it is also asserted that liquid containing viruses may by filtration be obtained free from proteins as determined by chemical tests. The uncertainty of such statements based on qualitative tests becomes obvious from the following considerations.

On filtering colloidal solutions such as ferric oxide or Prussian blue through filters that apparently retain these solutions completely it is not uncommon to find on the further side of the filter minute spots showing the color of the substance filtered. It is evident that the substance has passed the filter in amounts too small to be detected by ordinary tests. Viruses can be detected in very minute amounts mainly because they multiply under favorable conditions. Leucemic plasma may give rise to leucosis in amounts of 0.000001 cc.; it requires much larger amounts of plasma to demonstrate the presence of pro-

teins by ordinary chemical tests. If a filtrate contains less than a certain per cent of a single colloidal substance filtered (*e.g.* 0.1 per cent in the tests of Krueger and Ritter) the filter is arbitrarily designated as impermeable to this matter. Such determinations may be sufficient for a crude separation of colloidal substances or for the determination of the average size of the pores of collodion filters but are inadequate for an estimation of the size of viruses.

These difficulties can be only partly overcome by determining the concentration of the agent before and after filtration because the effectiveness of the agent as shown above may be increased by filtration. Moreover complete obstruction of the smaller pores of the collodion filter may occur leaving only the coarser pores open; through such a filter the passage of liquids is slow, and the agent may not undergo great dilution.

The uncertainty of estimates of the size of viruses on the basis of filtration tests may be illustrated by the following: The agent of Rous tumors was usually retained by the fine silicious filters (W) employed by Rous and from this it would seem larger than the majority of viruses; but Mendelsohn, Clifton, and Lewis (22) found that the agent passed collodion filters which retained proteins. They estimate the size of the agent to be from 15 to 50 $m\mu$.

SUMMARY AND CONCLUSIONS

The agent transmitting leucosis readily passed all types of silicious filters. Filtration is particularly successful when the plasma is freed from particles and substances that would otherwise obstruct the pores of the filter. Filtration through fine filters seems to be facilitated by preceding filtration through coarse filters.

A comparison of the periods of incubation of leucosis produced by unfiltered plasma and plasma passed through silicious filters shows that as a result of filtration, the incubation periods are somewhat prolonged. This suggests a slight or moderate decrease in the concentration of the transmitting agent in the plasma caused by filtration.

Filtration tests through collodion membranes indicate that the agent transmitting leucosis is much smaller than the virus of bovine pleuropneumonia (250 $m\mu$) and that it approximates the size of bacteriophage.

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