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FIFTH MONTHLY PROGRESS REPORT

OF RESEARCH

CARRIED OUT BY THE

LOVELL CHEMICAL COMPANY

at

WATERTOWN, MASSACHUSETTS

FOR

THE BIOLOGICAL DEPARTMENT, CHEMICAL CORPS, CAMP DETRICK

ON CONTRACT DA-18-064-CML-2407

for the period:

September 26, to October 24, 1953

Copy No. 3 of the Oct. 1953 Progress Report.

Twenty (20) pages.

The activities for this period have been concentrated on the evaluation of membrane developments to date as well as considerations for future investigations. The discussions are presented in the following form: preliminary assays of coarse type filters with inert particles; Aerosol chamber assembly; experimental membranes produced and plans for use of the membranes; and pertinent discussions related to the current investigations under the specific objectives outlined in the Contract Scope.

Determination of inert particle filtrations in coarse type filters by a method discussed in the Fourth Progress Report, page 4, has raised new questions and problems. Apparently the major problem lies in verifying particles, and their sizes, as a direct consequence of passage or retention through a given membrane. Due to outside contamination and suspected "backing plate retention", the present data is not accurate and indicative of the test membrane performance.

Fine mesh wire screens are being provided in the hope of eliminating backing plate retention. Other contamination is traceable particularly to small inert particles adhered on the surface of the control HA membrane. Complete removal of these outside particles prior to the test filtration has not been entirely successful. In the sizing determination under the microscope these various inert particles are not distinguishable.

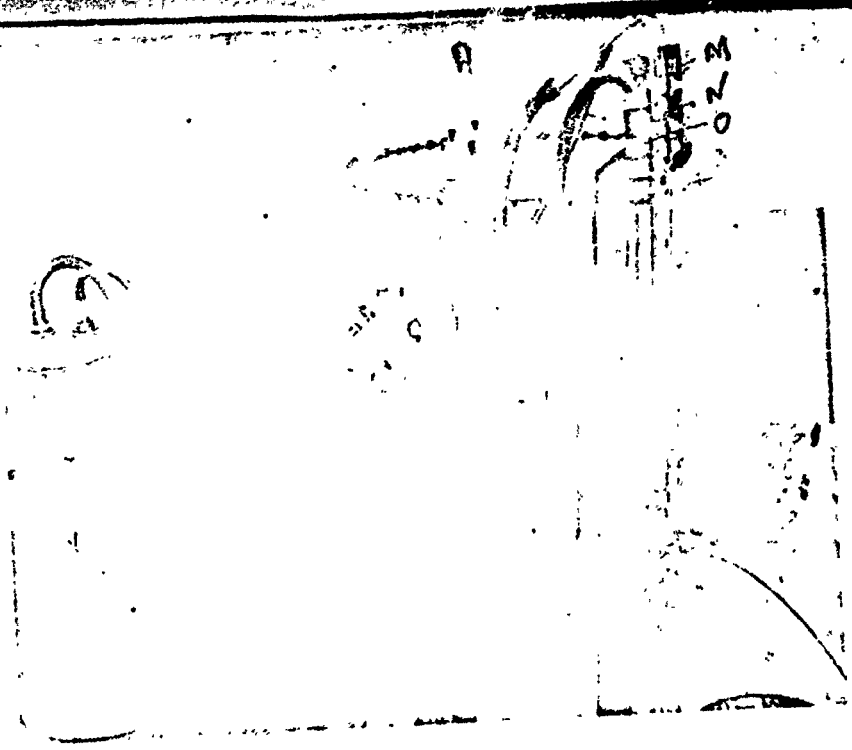
The approach and improvements to this problem are as follows: Oil immersion observation of the standard HA membrane with the collected inert particles on the surface was tried in a phase microscope. When polarized light was used the Fly Ash particles produced a shadowing effect distinguishable from other contaminating particles present. The best effects were apparent at 430X when the polarizing lens were not fully crossed.

Other particles of the desired size range have been made available by the Georgia Kaolin Company of Dry Branch, Georgia. These Kaolinite particles have flat hexagonal shapes with possible dimentional variations in two planes. By staining these particles with methylene-blue it may be possible to make them even more easily distinguishable on a control membrane without necessarily using phase microscopy.

Membranes tested by the above modifications so far indicate the presence of particles on the control filter up to a size range of 3.5 to about 5 microns. However due to contamination and possible loss of particles in the backing plate these figures are not reliable under the conditions of the test.

Aerosol Chamber Assembly:

It seemed most effective to present the detail of an aerosol chamber setup by use of suitable photographs. The three illustrations on pages 3 and 4, are accompanied by a descriptive legend describing each essential part of the chamber assembly. These will also be used as identifying references in any later reports pertaining to aerosol



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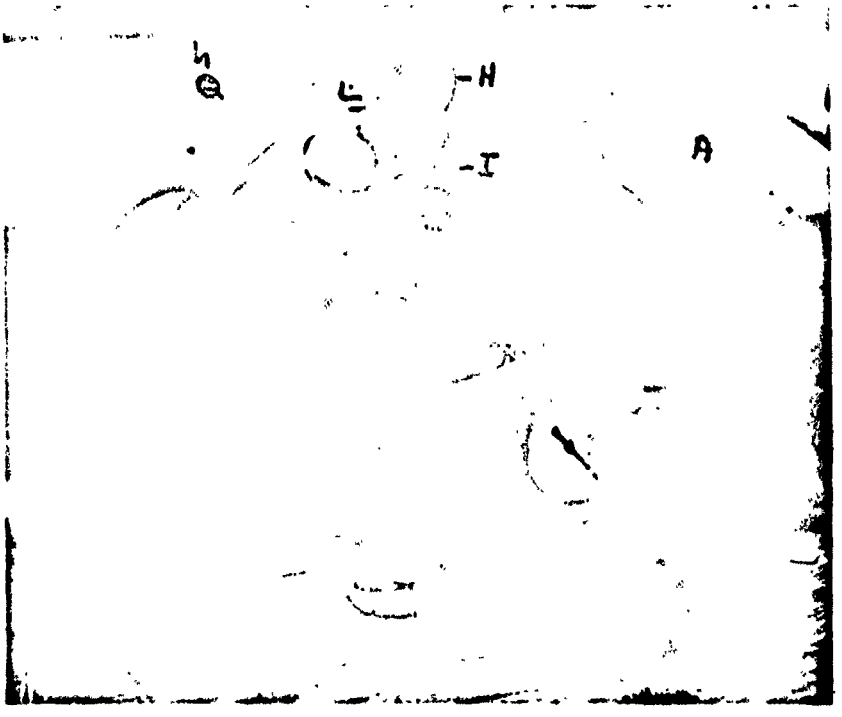

Figure 2: Closeup showing the sampling accessories. Sampling outlets from chamber at H,I. During sampling the membrane is secured in position L.

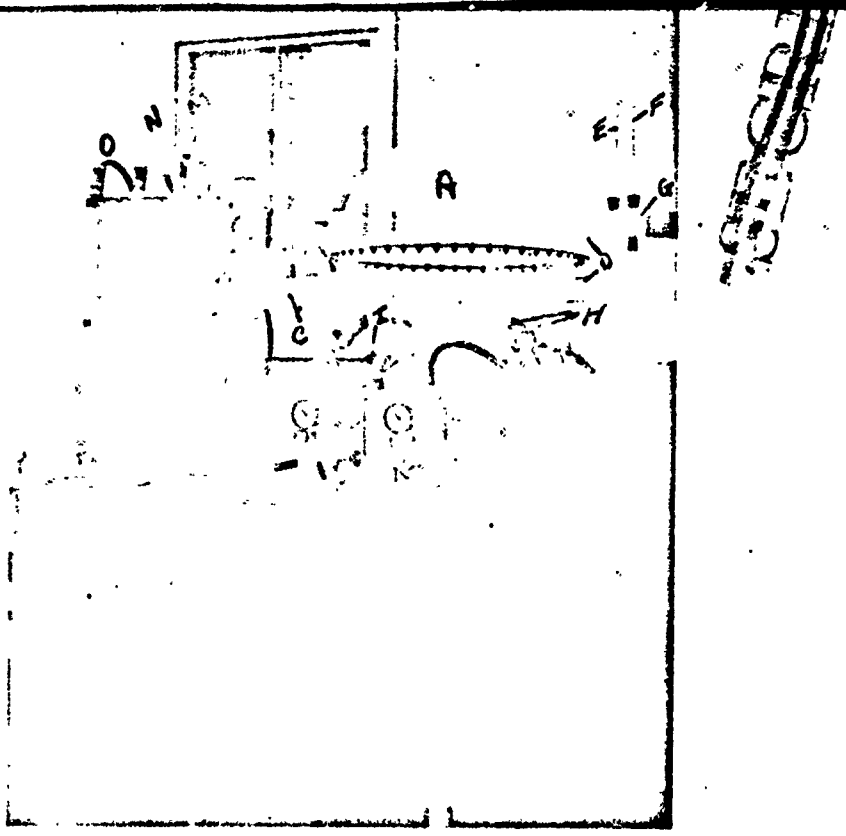

Figure 3: Side view showing overall ... 47.
Alternate inlets into chamber at Q. Four sampling outlets around the chamber at H. Outlet from chamber to outside atmosphere or special filters at G.

Legend:

A. Plexiglass sphere.
B. Secondary mixing chamber.
C. Baffles.
D. Outlets, alternate.
E. Dry bulb thermometer.
F. Wet bulb thermometer.
G. Outlet tube to atmosphere or special filters.
H. Aerosol membrane sampling outlet.
I. Impinger sampling outlet.
J. Impinger and flask for controls.
K. Vacuum lines.
L. Burt-Goetz aerosol assay unit.
M. Flowmeter-atomizer air supply.
N. Flowmeter, dry air.
O. Flowmeter, moist air.
P. Compressed air supply.
Q. Inlets, alternate aerosol.
R. Chicago atomizer.
S. Air humidifier.
T. Reservoir for fluid to be atomized.
U. Mixing chamber for Chicago atomizer.
V. Sparger.

Note: Electro-dryer or silica gel air-dryer not shown.

The fluid to be atomized is placed in reservoir T. Air metered by the flow-meter M is passed to primary air flow of the Chicago atomizer causing dispersion of fluid in T. The mist formed passes to the mixing chamber U, where it is mixed with metered dry and wet air in the proper proportions and then proceeds through the baffles C, to sphere A. Here samples are taken with a membrane at H, and impinger at I. Excess aerosol and air passes out at D past wet and dry bulb thermometers E and F.

Humidification is achieved by bubbling air through the sparger V, in bottle S.

Drying is accomplished by means of an electrodryer or by passing through a column of activated silica gel placed in line M (electrodryer or silica gel column not shown). Further comments on the aerosol detection problem will be made in Part III.

The steam autoclave was received in this laboratory during the early part of the month. Installation has been completed and its operation meets our requirements ideally.

PART II: Experimental membranes produced and the r testing.

As noted in the Fourth Progress Report, page 9, other types of cellulose acetate and acetate-butyrate have been evaluated in membranes. Cellulose acetates include the following and are to be added to those listed in Table I, Third Progress Report, page 8.

1.	55.7 to 57.0	2 to 4
2.	54.9 to 55.9	8 to 15
3.	55.4 to 56.2	17 to 33

From the point of view of combined acetic acid, or substitution, these cellulose esters are intermediates [illegible] previously investigated. Their major variations are seen to be in viscosity differences. As expected by the rule of substitution which we have previously discovered and mentioned, the above three esters did not produce membranes of Z values beyond limits already found. They are however, relatively high in acetyl substitution and their physical properties permitted an evaluation of this latter property. As observed in previous experimental membranes the wetting properties of a membrane appear to be proportional to the viscosity of the original cellulose ester. When other factors are held constant, membranes are generally faster and more uniformly wetted when the viscosity of the original cellulose type is low. Likewise, high viscosity esters yield much poorer wetting characteristics or lack of uniform wetting.

Moreover, analysis of all membrane data concerning cellulose acetate viscosities strongly indicates effects on certain other properties. Considering the cellulose-nitrate type and viscosity employed along with cellulose acetate, for example, when these viscosities are chosen at wide variances (in the same solvent and solids content) either the cellulose acetate viscosity being higher or lower than that of the cellulose-nitrate, wide variations in film properties result. This applies particularly to uniformity of wetting, Z values, smoothness of film surface

and generally lines, patterns and strie on the film surface, as well as generally increased variance in bacteria growth characteristics on membranes. Specifically, for a given cellulose acetate type suitable in a given pore size membrane the corresponding cellulose nitrate viscosity should be employed in order to maximize general membrane uniformity.

In binary sol preparations a narrow range of viscosities of both cellulose-nitrate and acetate are also important. The homogenizing process of final sol preparations enhances this "uniform chain breakdown".

It may be pointed out however that these sol preparations of cellulose esters are typical polymeric systems and therefore not exactly reduceable to sharply defined properties at all times. From the background of membrane production data and experiences in the laboratories of this company, these statements are well borne out. Additional factors recognized to effect the operation of preparing and casting membranes are (1) control of temperature of the entire mix and evaporation of solvents during homogenization. An optimum temperature and cycle of homogenization is indicated during preparation-- an essential factor contributing to reproducibility. (2) Environmental conditions existing during actual membrane casting. For instance, the ambient conditions of humidity and temperature, and, possibly atmospheric pressure. In the case of finer type membranes empirical considerations suggest that these effects are more pronounced on membrane structure formation during the critical gelation stage. Perhaps a practical route leading away from these undesirable and variable effects, lies in casting a smaller size

sheet when fine membranes are involved.

However these aspects remain to be further considered at a later date pending final conclusions on phage testing and retention obtained. Relative to conditions (1) and (2) above, a worth-while approach lies in certain ageing factors involving cellulose lacquers. This will be brought out presently in more appropriate discussions.

Membrane preparations and testing were also completed on the following acetate-butyrate in binary mixture with cellulose-nitrate. These are additional to those listed in table I, Second Progress Report, page 10. They are;

	% Butyryl	% Acetyl	Viscosity
1.	17.5	29.5	1.5 to 2.5
2.	17.5	29.5	10 to 20
3.	17.5	29.5	33 to 35

It will be recalled that these acetate-butyrates types are now commercially available whereas the 16% butyryl, 31% acetyl type has been discontinued. Membranes prepared from the above acetate-butyrates show no shrinking tendencies on the glass plate for a wide range of Z values. These membranes are also comparable to those prepared with the previous 16% butyryl, 31% acetyl type ester in flow rates and wetting properties. Generally formulations with cellulose acetate-butyrates yield membranes of higher flow rates, longer wetting times and increased brittleness as compared with cellulose acetate prepared membranes. Various Triton-type wetting

agents (non-ionic) of greater and lesser water, organic solvent solubility formulated in these experimental membranes produced no marked effects on membrane wetting properties. These acetate-butyrate membranes were also exposed to ammonia vapor in the hope of rendering these films hydrophillic. After brief exposure the non-wetting characteristics were practically unchanged. After several hours exposure the membranes turned yellow, wettable and very brittle.

The effect of viscosity with acetate-butyrates is analogous to that described above with viscosities of cellulose-acetates. However in the case of membranes prepared from low viscosity acetate-butyrates the effect of brittleness is more pronounced. In summation, these effects and properties with acetate-butyrate prepared membranes detract from their suitability and applications as hydrosol type membranes. Their further evaluation can therefore be by-passed in favor of more promising membranes.

From the point of view of cellulosic materials, studies to date have greatly amplified the understanding of mechanisms in membrane formation and properties. The acetate-butyrate and acetate esters of cellulose investigated represent all commercially available[1] types soluble in acetone. These cellulosics have been evaluated essentially on presently developed techniques and equipment. Further general discussions of these conditions will be made in art III.

[1] As determined by direct contact with prime manufacturers of cellulose derivatives in the United States.

Other cellulosic esters [illegible] investigated are the new cellulose acetate-sorbate and certain cellulose-nitrates. The cellulose- acetate-sorbate suggests possibilities of cross-linkage with entirely different monomeric type materials. Some of these trials are underway and will be summarized in a later report.

In the Fourth Progress Report, pages 8,9, four separate steps were outlined as qualitative approaches in membrane studies involving cellulose esters. Trials made and conclusions will be given on those four aspects separately:

I. Miscible additives to cellulose-nitrate:

Strictly speaking these are legion but upon further consideration of a solvent-diluent system several materials are ruled out. From such empirical considerations the following materials and results were obtained. Considering formamide, only just now commercially available, and a solvent for many classes of compounds namely; cellulose-acetate, certain proteins and saccharides; miscible in water, glycerol and organic solvents, and a softening action on cellulose-nitrate, the following trials were made in binary sols:

A. Dissolved in formamide were proteins such as Kelcote and casein; a saccharide namely, starch; and cellulose-acetate. The significant result is that these materials produced membranes within limits already found considering flow-rate and retention. At increased concentrations all the formamide additions become unstable, tending to separate out of suspension, and producing the

effect of brittleness and reduced tensile strength on the film.

B. Addition of de-waxed, alcohol-soluble shellac along with cellulose-acetate and nitrate generally increases the brittleness of membranes. Increasing amounts of shellac increases the porosity of the membranes (lowers the Z values) accompanied by increased brittleness and slightly colored line patterns in the resulting membrane.

C. Other miscellaneous additives to cellulose-nitrate evaluated were these:

(a) Alginic acid, whic basic structure is analagous to cellulose, imbibes large amounts of water. During gelation and at annealing temperature this water would presumably take part in the pore structure formation. Such formulation however exhibits very high film shrinkage during gelation though porosity is apparently retained. Because of shrinkage these films could not be extracted or otherwise tested at the present time.

(b) Alcohol-water soluble nylon resin was also added to cellulose-nitrate with the usual solvents - diluents. High shrinkage during gelation resulted (in similar fashion to (a) above). These films also retained porosity after shrinkage but again due to this shrinkage they were not capable of being extracted or otherwise tested at the present time.

The reason for this was to establish a relative standard by which comparisons could be made. Results of these brief experiments definitely show variable effects on membrane properties. However due to limited data the pattern of the effects is not clear, the complexities not being capable of definition within the time scope of this contract.

Generally a mixture of organic liquids with solvent or co-solvent power on cellulose-nitrate and acetate accompanied by a low dielectric constant for each liquid produces a membrane with a higher flow rate. If the solvent or co-solvent (distinguished from a 'diluent only' material) has a low vapor pressure (slow evaporation) there is a point at which the membrane is blushed but not porous and another point at which the film becomes completely transparent. Typical effects of diluents are impossible to single out from the present data. Also the presence of water in the formulation alters the above general relationships.

From these experiments the presence of water in a formulation can be related to a definite property of a membrane. In the complete absence of water, anhydrous solvents and diluents of a sol preparation, such membranes can be porous but are almost completely hydrophobic.

The above experiments on solvents and diluents have indicated that the flow rate of the 'HA type membranes' can be increased by a factor of about 4 without significantly distorting other properties of the membrane. The immediate

significance of these results will be to apply them to fine membranes under present developments.

III Gelation Atmosphere:

There have been no investiagtions on developments of this qualitative aspect to date. Considerations indicate however that these conditions are related in section II. above.

Variables presumably affecting membrane formation under this classification are; temperature, moisture, atmospheric pressure, and air flow in the surroundings of a film from the casting operation to the end of the gelation cycle. Regarding evaporation of solvent-diluent systems these variables are related to heat effects of evaporating liquids, azeotropic mixtures, and generally the rate of the gel structure formation.

These effects suggest that membrane film uniformity or pore size control factors are achievable by these means. However, the equipment and sensitive controls required for these experiments are considerable, and are not contemplated at this time.

IV. Ageing effects of additives in sol formulations:

Experiments have been initiated for the evaluation of ageing additives in membrane formulations. Since these trials involve various time periods no results are available for reporting at this time.

The literature indicates that in a solution of cellulose-nitrate in acetone, the nitrate groups are solvated specifically by acetone. Remaining hydroxyl groups must therefore be solvated by alcohol co-solvents. Such

a co-solvent has the well-known effect of reducing the solution viscosity of cellulose esters containing unsubstituted hydroxyl groups.

In as much as cellulose-nitrate lacquer presently available is wet with alcohol, solution viscosity is relatively high at the solids presently utilized, and ageing periods for membrane preparations are presently established at a thirty day minimum from the date of lacquer manufacture. These considerations seem appropriate: the nitrate group on cellulose as indicated in the literature is an ester group not a nitro group as the common name (nitrocellulose) implies. It is therefore hydrolyzable. Some hydrolysis of nitrate particularly in the presence of water is entirely possible.

During homogenization of sol preparations when the initial cellulose solution viscosities are high (before sol II addition takes place), temperature increases are encountered. These conditions become more favorable for hydrolysis to take place. Once started the presence of free-acid in a sol mixture catalyzes the reaction even more. Theoretically then, there is a maximum temperature at which hydrolysis becomes appreciable and an optimum temperature at which sol preparations should be homogenized. These effects are conceivably related also to membrane uniformity, reproducability, and properties. Furthermore since fine membrane sols are generally more viscous these effects become more critical and a highly uniform dispersed system becomes more difficult to attain.

Bacteriophage Notes:

Basic phage testing of membranes has continued

The suspicion that a "loading factor" might be involved in filtration of phage through a membrane appears to have been confirmed. By loading factor is meant the apparent relation between amount of phage added to the filter and the amount recovered after passage. With a small 'load', about 10^9 infectious particles, variable recovery was obtained with passages as low as 10^{-5} % in some cases. With a heavier "loading" as mentioned below, this apparent variation seems to have disappeared.

It was found that loading with ten times as much phage (10^{10}) appears to give reproducable recoveries which were much greater than previous ones. This is now being confirmed. Consequently such loading factors will require evaluation with regard to retention. The effect appears that certain membranes are less retentive than initially indicated. It is also important to note that this data is contingent upon the dual donditions of phage particle sizes and membrane uniformity itself.

The initial bacteriophage (our designation DT-3) was tentatively reported by Camp Detrick to have an apparently wide range of sizes. Plating shows considerable variation in plaque sizes. Three substrains with three different plaque sizes have been separated. These strains are still being selectively plated and now appear quite uniform within the strain. This indicates that the range of size of the phage should be smaller.

Strains of Coliphage T-3 have been received from Dr. Weigle at Cal. Tech. , Dr. Hershey at Carnegie Inst., Cold Spring Harbor, Long Island.

Strains of Coliphage T-5 have been received from the American Type Culture Collection, Dr. Hershey and Dr. Weigle. The T-3 and T-5 strains are in the process of building up and selection.

During Dr. Bolduan's visit to this company on October 6th and 7th 1953, it was concluded that it would be advisable to further check the phage size using phage produced in the presence of a synthetic medium. This would make identification of the phage particles in the electron microscope more definite.

To that end this laboratory would work out the details of the medium and its application to the phages. In coordination with fine membrane developments, Mr. Krabek would take the trip to Camp Detrick to collaborate in the use of the electron microscope for sizing of the phage particles and eventual membrane evaluation.

In the course of the discussions with Dr. Bolduan it was also agreed that the use of serological methods for identification of the phages would be explored to aid in identification of phages on hand.

PART III

It seems very appropriate at this stage of investigations for this contract to review the course of our work specifically under the objectives of the contract requirements. These discussions may be conveniently grouped in their order of priority, namely:

1. Fine membranes.
2. Coarse membranes.
3. Aerosol detection of viable organisms.

1. Our studies of the mechanisms of membrane preparation have shown that the amount of subtitution on cellulose-acetate is a direct factor in controlling the flow-rate of membranes. These low flow-rate membranes in turn are being evaluated with phage filtrations in order to establish retention , and a relation between flow-rate and retention. During the course of the phage assays, experiments are continuing in part along the lines of maximizing the uniformity and reproducibility of these fine membranes.

The investigations with cellulose-acetato-butyrate and cellulose-acetate types are now complete insofar as commercially available types are concerned. Cellulose-acetate types formulated thus far indicate superior all-around characteristics for a millimicron retention hydrosol type membrane over the acetate-butyrate types.

The cellulose-acetate type applicable for this purpose has a combined acetic acid content of 56.1 to 56.6%. Since cellulose triacetate comes as high as 61.5 to 62.5% combined HAc, from the substitution the triacetate would be highly indicated. This is not particularly feasible for these reasons: The cellulose of 56.1 to 56.6% HAc is the highest substituted acetate ester still soluble in acetone solvent. The triacetates are not soluble in acetone or even ester solvents but require high percentages of chlorinated solvents. There are then three basic reasons for ruling out triacetate in membranes. (1) Chlorinated solvents are highly toxic. (2) Triacetates, which contain virtually no hydroxyl, are indicated to produce brittle membranes. (3) Use of chlorinated solvents would also have

questionable effect on microorganisms in general membrane uses.

The nature of the studies conducted thus far in search of fine uniform membranes have been feasable and practical insofar as equipment and materials are concerned. These studies for the most part have involved an analysis of recognized variables on which definite quantitative data has never been recorded or available. In the absence of such data which must have accompanied previous membrane developments, the effects or possibly the limitations of a given variable were completely unknown. Consequently the pattern of our investigations thus far are justifiable on the basis of mechanisms leading to the particle size retention.

A further approach to these studies particularly on the fine membranes down the line to extreme fineness, lies in basic analyses of solvation and solution processes of colloidal cellulose ester dispersions. Data on this general subject is becoming more abundant in current literature. It is in this basic approach that dielectric-constant equipment and measurements were calculated within this program of investigations. However in view of the short term of this contract it has now been decided that this type of basic study will not be initiated.

Theoretically, membranes of graded porosities in the colloidal dimension range approach the regions of highly developed molecular arrangement and symmetry of structure. In these aspects it is not unreasonable to consider the nature of chemical bonds and the critical requirement of a definite macro-molecular arrangement in a very fine membrane structure. It becomes equally important to produce a given fine porous structure as it is to produce these pores of a

very uniform size.

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2. In the search of developing coarse type membranes cellulosic lacquers have not indicated a single clue leading to pore size dimensions approaching ten and twenty microns. This strongly suggests, therefore, that we direct experiments more objectively in accordance with specific contract requirements. For these reasons we take the position that new basic ingredient developments (outside of cellulose) be initiated at this time. This sort of development conceivably encompasses the consequence of fine type as well as coarse type filtering materials.

3. In view of project priorities, extensive testing and evaluation of present membrane developments, and the increasing scope of both fine and coarse type membrane studies, work concentration in the foreseeable months will be devoted to these problems. The aerosol detection study is likewise a broad basic type of investigation and time allotment on this part of our project is secondary to the membrane developments. This point was discussed during Dr. Bolduan's visit to Watertown and was mutually agreed upon.

As illustrated in Part I the basic physical assembly of the chamber has been accomplished. In accordance with developments in sections 1 and 2 above, time allotment to the aerosol chamber operation will be subject to discretion. The chamber is now available when that opportunity presents itself.

Respectfully submitted,

LOVELL CHEMICAL COMPANY

Roger G. Finette

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Project Director