TABLE 1

COMPLEMENT-FIXING REACTIONS OF THE SHEEP PNEUMONIA VIRUS WITH HOMOLOGOUS AND FELINE PNEUMONITIS ANTISERA

Serum	Highest dilution of antigen in which complement was fixed in presence of serum		Highest dilution of serum in which complement was fixed in presence of antigen	
	Infected yolk sac	Normal yolk sac	Infected yolk sac	Normal yolk sac
Sheep serum				
Preinfection	1–20	1–10	< 1–4	< 1–4
Postinfection	1–50	1–10	1–32	< 1–4
Feline pneumonitis antiserum				
Kitten "S"	1–40	1–10	N.T.*	N.T.
Kitten 236	1–50	1–10	1–16	< 1–4
Normal kitten serum	1–10	1–10	< 1–4	< 1–4

* N.T. = Not tested.

monitis antisera. Sera of the 3 kittens mentioned above were also tested for complement fixing antibodies to the sheep lung agent. These results were negative and do not appear in Table 1.

In the qualitative test, undiluted through eightfold dilutions of the 10% antigen suspensions were incubated in a water bath for 2 hr at 37° C with a constant (1–8) dilution of each serum specimen and 2 units of complement. After adding a sheep red blood cell suspension which had been sensitized by the addition of 2 units of hemolysin, the tubes were reincubated and the tests read when the control series exhibited the appropriate reaction. Readings were again made after the tests had been held overnight at 4° C. The quantitative test was conducted according to the above procedure, using serial twofold dilutions of each serum specimen and 2 units of antigen. Complete, rather than partial fixation, was selected as the end point. The titers shown in Table 1 refer to the initial dilution of the antigens and the sera, respectively, before the addition of the other reagents.

An elementary body agent has recently been incriminated as the cause of enzootic abortion in ewes in Scotland (*4*). It was demonstrated subsequently that this virus is related antigenically to both the psittacosis and the lymphogranuloma venereum viruses (*5*). The sheep pneumonia virus and the virus of enzootic abortion in ewes are, therefore, undoubtedly related antigenically. However, the agent described in this paper was isolated from individuals in flocks in which there was no evidence of the existence of enzootic virus abortion. Furthermore, the fact that the sheep abortion virus was found incapable of producing pneumonia in either sheep or calves (*4*) is evidence that the two agents isolated from sheep are distinct viral entities.

On the basis of our observations on its host specificity, tropism, and antigenic relationship with the feline pneumonitis virus, it is tentatively concluded that the agent isolated from sheep lung is a new member of the psittacosis-lymphogranuloma-pneumonitis group of viruses.

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Some Aspects of the Phenomenon of Coacervation

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The name coacervation was given by Kruyt (*1*) to the phenomenon of limited solution observed with a large number of colloids and colloid mixtures. A coacervate is thus regarded as a colloidal solution which is immiscible with excess solvent. Bungenberg de Jong (*2*) made an extensive series of investigations along this line and attributed this phenomenon to the separation of extensively solvated colloidal particles out of the solution by the addition of some nonsolvent or precipitant, solvation being due to the electrostatic attraction of the charged colloidal particles. This idea was widely prevalent till 1940, since the recorded data on coacervation were confined mostly to the electrically charged colloidal particles in aqueous solution—e.g., gelatin, gum arabic, starch, etc. In 1942 Dobry (*3*) showed that coacervation can take place in nonaqueous media with high molecular weight substances, which are generally molecular colloids. Thus, in precipitating cellulose acetate and polystyrene out of a good solvent by the addition of a suitable nonsolvent as precipitant, Dobry many times obtained separation of coacervates rather than granular precipitate. We have also observed that in precipitating polymethyl acrylate out of methyl ethyl ketone by the addition of methyl alcohol a viscous liquidlike mass separated out, which on prolonged standing shrank and left a film of polymeric substance at the bottom of the container. Thus it became evident that an explanation based on the electrical charge of the particles should be revised in order to accommodate within its purview cases of uncharged particles as well.

Bungenberg de Jong (*4*) has recently put forward a more general explanation of the phenomenon of coacervation. It is to be noted that until now almost all the substances that can form coacervates are high

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molecular weight chain compounds, be they in aqueous or in nonaqueous solution. Bungenberg de Jong regarded coacervates as macromolecular aggregates in which a considerable amount of solvent is immobilized or occluded inside the loops of molecular skeins. This idea also gets some support from the work of Alfrey, Bartovics, and Mark (*5*) on the solutions of polymers in good and bad solvents. It has been shown by these authors that the molecules of polymeric chain in the dissolved state in good solvents take up an extended structure. As the solvent character is rendered more and more unfavorable by the addition of some precipitant the polymer chain becomes more and more coiled up. So it is expected that at the time of separation the extensively coiled-up polymer molecules will carry along with them considerable quantities of occluded solvents immobilized inside the loops of molecular skeins. The separation of coacervates therefore follows from the idea of flexibility of the chain molecules and the solvent immobilization inside the coiled-up molecules. It follows as a corollary from these viewpoints that if a molecule separates out of the solution in its extended configuration it will give a solid product, whereas if it separates in the coiled-up state coacervation will take place.

In order to test this point experiments were planned taking into consideration the present conception of the change in the molecular configuration of polyelectrolytes under different working conditions (*6*). The best-known example of the coacervation phenomenon is the separation of gelatin from aqueous solution by the addition of alcohol. Gelatin is a protein that contains a number of carboxyl and amino groups distributed along the chain. When sodium hydroxide is added to a gelatin solution some of the COOH— groups are converted into COONa, which in its turn undergoes dissociation into COO^- and Na^+. The protein chain is thereby left negatively charged, and the repulsion between the similarly charged COO^- groups on the same chain will cause the protein molecule to take up an extended configuration. If alcohol is added to the solution, precipitation will take place more or less in the solid form. Similarly, when HCl is added to the solution, the NH_2 groups will be converted into hydrochloride, which will also undergo dissociation, leaving the extended chain positively charged, and the precipitation will lead to solid separation rather than coacervates.

If, however, sufficient sodium chloride is added to solutions of gelatin hydrochloride or sodium gelatinate at different pH's, the dissociation of sodium gelatinate or gelatin hydrochloride will be considerably suppressed, owing to common ion effect; the negative and positive charge centers will be neutralized, and the molecules will take up a less extended, average coiled-up structure. Addition of alcohol to such solutions will lead to coacervation rather than solid separation. The results of precipitation of gelatin from aqueous solution at different pH's by alcohol in the presence and in the absence of sodium chloride are summarized in Table 1.

TABLE 1

PRECIPITATION OF GELATIN BY ALCOHOL

Without NaCl		With NaCl	
pH	Nature of the precipitate	pH	Nature of the precipitate
9.6	Granular solid	9.8	Viscous liquid
8.7	Hard gel	8.5	" "
7.1	Viscous liquid	7.5	" "
5.5	" "	5.7	" "
4.6	" "	4.0	Setting to gel
3.5	Granular solid	3.0	" " "
2.6	" "		

It is evident from Table 1 that in the absence of sodium chloride gelatin separates as granular solid both at higher pH's (where the conversion of gelatin to its sodium salt is nearly complete) and at lower pH's (where gelatin exists as hydrochloride) by the addition of alcohol, whereas in presence of sodium chloride coacervation takes place at all pH's (pH adjustments were done in these cases either by the addition of NaOH or HCl).

In the case of precipitation of gum arabic from aqueous solution with alcohol, similar results have been obtained. At higher pH's separation takes place in the solid form; and in the presence of sodium chloride at those pH's separation of oily droplets (visible under the microscope) takes place. The droplets gradually agglomerate to a sticky, jellylike mass.

These observations, along with the analysis set forth, therefore lend further support to the Bungenberg de Jong explanation of the coacervation phenomenon as due to separation of coiled-up molecules with a considerable amount of immobilized solvent inside the loops of molecular skeins.

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Effect of Heat on the Properties of Urea at its Melting Point

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The melting point of pure urea is 132.7° C as given in the literature (*1*). If urea is heated above its melting point at temperatures from 150° to 200° C, biuret, tricyanurea, and several other products are formed successively. Some of them are generally considered to represent polymerization products of the single