

concentration, and titer of the virus, it was estimated that 1000 to 10,000 particles of yellow fever virus are necessary to produce infection.

These results and the observation that antibodies appear in monkeys so early in the course of infection make it seem highly probable that most of the virus particles present in the circulating blood and responsible for the boundary were already neutralized.

**Comparative Studies on Ultracentrifugation and Serological Reactions of Bacteriophages, Plant Viruses and Insect Viruses.** PROF. DR. ANDRÉ GRATIA (*Liège*).

In 1933, the first air-driven ultracentrifuge of Henriot and Huguenard built by Bardet in Paris was installed in our laboratories in Liège.

Certain mechanical difficulties having been overcome, technics we have devised allowed us to study easily sedimentation of bacteriophages, plant-, insect- and animal viruses, bacterial toxins and enzymes, normal and immune serum, antibodies, alexin, etc.

With Paillot we have obtained by differential centrifugation of serum of silkworm affected by jaundice the isolation of polyhedral bodies and of minute granules to which virulence is bound. Polyhedra are made up of virulent granules which aggregate themselves in a crystallin form of the cubic system. Granules and polyhedra have an identical antigenic constitution which is entirely distinct from normal silkworm tissues and from other insect viruses proving they are specific and autonomous individualities of a probably parasitic and exogenous origin.

Sedimentation rate of several phages was studied. Most of them have a rather similar one, some have a much slower one. When the latter are centrifuged together with the former or with tobacco mosaic virus they do not come down faster, thus showing no adsorption on other phages or on another virus. But when tobacco mosaic protein mixed with phage is crystallized, a small amount of phage is taken in and remains even after eight recrystallizations.

As adsorption of phages on sensitive bacteria was found to be completely inhibited by convenient amounts of electrolytes—this inhibition depending on the valence of both anions and cations—and as adsorption might take place or not according as pH is acid or alkaline, it can be easily avoided during ultracentrifugation if proper electrolytic and pH conditions are provided which differ for each phage.

Inactivation of phages was found to occur at a definite concentration of certain salts (Na, K, etc.) and also below a certain pH which might vary

according to the kind of phage between pH 6.0 and pH 3.0. As ultracentrifugation increases those harmful effects as well as others, it is useful to determine for each phage, virus or other agent, the most favorable medium conditions to avoid inactivation during ultracentrifugation.

By differential ultracentrifugation of Twort's vitreous material, phages could be concentrated as high as  $10^{13}$  in a rather purified state.

*Discussion by* PROF. DR. ANDRÉ GRATIA (Liège):

As answers to Dr. Tiselius' questions I will say:

(1) In certain cases quick freezing of the tubes after centrifugation was used. Although convection currents could be feared, titrations did not show any appreciable disturbance of the layers.

(2) In cases where sediments were sticking to the bottom of the tubes, we did not find difficulties in having them put back in suspension, with one exception: a fibrin-like sediment in serum of normal silkworm.

The following also participated in the discussion: PROF. DR. ARNE TISELIUS (Upsala), DR. J. H. BAUER (New York), and DR. W. M. STANLEY (Princeton, New Jersey).

**Experiments on Immunity to Fowl-pox, Especially by Means of Skin Grafts on the Chorio-allantois of Chick Embryos.** DR. ERNEST W. GOODPASTURE and MISS KATHERINE ANDERSON (*Nashville, Tennessee*).

The mechanism of acquired immunity to virus infections is as yet but poorly understood. Although after recovery from any one of several diseases of this type the host becomes completely resistant to reinfection and the blood serum acquires the capacity to neutralize the respective virus, there is still a question whether or not the susceptible cells themselves participate in this immunity.

We have investigated this problem by the use of a new technique, namely, the grafting of normal and immune chicken skin upon the chorio-allantois of developing chick embryos and then inoculating the grafts with fowl-pox virus. Our experiments have shown that the cutaneous epithelium, which is completely refractory to fowl-pox infection while a part of the immune host, becomes quite as susceptible as the epithelium of normal chicken skin when grafted upon the chorio-allantois.

Chorio-allantoic grafts of normal skin, grafted upon muscle of normal chickens, is quite susceptible to infection with fowl-pox virus, but the susceptible grafts from immune chickens become insusceptible when grafted upon the muscle of an immune chicken.