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Virus Distribution in Reversible Soluble and Insoluble Phases of Neutralized Papilloma-Virus Protein.*

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The neutralizing effect of homologous immune serum on the rabbit papilloma-virus protein is quantitatively reversible[1] by simple dilution over the whole range[2] of serum-virus relations accessible to study by infectivity-measurement.[3] Under proper conditions of serum-virus proportions, precipitates occur in the mixtures, consisting presumably of virus and specific antibody. In the present study the distribution of virus between the soluble and insoluble phases in one region of serum-virus quantities has been determined.

The results reported here were derived from 8 correlated experiments with papilloma-virus protein and an antipapilloma rabbit-serum designated as D. R. 496. The findings of 7 experiments with this serum have been tabulated in detail elsewhere.[1, 2] An additional study made in the desired region of serum-virus relations is described here.

Papilloma-virus protein procured under standard conditions[3] by ultracentrifugation was mixed with undiluted immune serum D. R. 496 as previously reported[2] so that the final concentration of total virus was 24.4 γ per 0.1 cc (virus pI 4.6, serum pD 0).[1,2] After 1½ hours the precipitate forming at room-temperature was sedimented in an ordinary horizontal International Centrifuge No. 2 at full speed for 30 minutes. The clear supernatant fluid was carefully pipetted from the pellet which was then suspended in 0.9% NaCl solution to the volume of the supernatant fluid (4.5 cc). The supernatant fluid and the pellet-suspension were inoculated[3] in successive twofold dilutions in a group of 32 rabbits, a number statistically adequate[3] for the problem at hand. Twofold dilutions of untreated viral protein were inoculated in the same animals for standardization.

With the serum-virus relations described, the free virus of the

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1 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, 1941, **68**, 133.

2 Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., *J. Infect. Dis.*, in press.

3 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, 1939, **65**, 306.

whole mixture without separation of the precipitate is 3 γ per 0.1 cc as previously reported,[2] indicating neutralization of 21.4 γ of the 24.4 γ initially present. When the precipitate was sedimented, however, the free virus in the supernatant fluid is not 3 γ but 0.008 γ. Dilution of the pellet-suspension resulted in progressive freeing of virus until the seventh twofold dilution where the rate of change in free virus paralleled that of the untreated viral preparations containing equivalent amounts of total virus. From the relation of control and experimental regression lines[1, 3] in this region, the total virus present in the initial pellet suspension of 4.5 cc was found to be 6.1 γ per 0.1 cc. Of this, 0.9 γ per 0.1 cc was in free form. In the supernatant fluid, then, there was 18.3 γ total virus of which 99.1% was present as soluble neutralized virus. This represented approximately 75% of the total virus per 0.1 cc initially used.

A possible explanation of the above facts may lie in the following equilibria between virus and serum. Virus and serum mixed under the present conditions yield a system of soluble and insoluble phases of neutralized virus. With the system intact, *i. e.*, prior to centrifugation, equilibrium can be expressed as

$$\text{V} + \text{B} \rightleftarrows (\text{V.B.}) \rightleftarrows (\text{V.B.})\downarrow\dagger$$

It is known that the state of equilibrium for a given concentration of serum and virus is constant[1, 2] and that the equilibrium is reversible as shown by the dilution-phenomenon in the present and previous experiments.[1, 2] Under these circumstances the combined virus in both soluble and insoluble phases will contribute to the level of free virus in the intact system. When the precipitate is sedimented, however, the effect of the insoluble phase will be reduced by removing it from contact with the soluble phase, resulting in further combination of free virus and free serum to satisfy the equilibrium. Such is possible, for free virus exists in the uncentrifuged mixture in the amount of 3 γ per 0.1 cc, as determined by direct measurements[1, 2] and the intact system contained enough serum to neutralize a total of 27.7 γ virus per 0.1 cc in the presence of an optimal quantity[1] of virus, 44.9 γ per 0.1 cc. In the end, the level of free virus in the supernatant fluid will depend on the demands for equilibrium between free virus and the soluble virus-serum complex, and thus the quantity of free virus in the supernatant fluid is reduced below the level existing in the intact system. The amount of free virus found in the pellet-suspension diluted to 4.5 cc was related at least in part to dissociation by dilution, so that it cannot

†V—virus; B—antibody; (V.B.)—soluble phase; and (V.B.)↓—insoluble phase.

be said whether or not all of the virus precipitated was in the neutralized state.

These results provide direct evidence for the existence of both soluble and insoluble phases of neutralized virus and for the dissociation of both with dilution when the 2 phases are separated. The data likewise afford an estimate of the distribution of virus between the 2 phases separated in this way by centrifugation. It should be emphasized that the described distribution can be considered only for the special region of serum-virus amounts employed. The reduction in free virus in the supernatant fluid resulting from removal of the insoluble phase, a phenomenon already noted by others in studies with unpurified viral preparations,[4] could not have been due to sedimentation of free virus since it was not demonstrable in the pellet-suspension. The findings suggest the possibility of a shift in chemical equilibrium as an explanation of the phenomenon.

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Comparative Activity of Nicotinic Acid and Nicotinamide as Growth Factors for Microörganisms.*

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Investigations of the past few years have demonstrated that a number of microörganisms must be supplied with either nicotinic acid or nicotinamide for continued cell multiplication. When these compounds are supplied in excess no difference in activity is noted, but when suboptimum quantities are used a quantitative difference in activity is often encountered.

While studying the nutritive requirements of the Pasteurella group of bacteria, it was found that nicotinamide could *not* be replaced by nicotinic acid in the cultivation of those members of the Pasteurella group which cause hemorrhagic septicaemia in animals. In a basal medium of hydrolyzed purified gelatin, supplementary amino acids, glucose and inorganic salts, growth of many strains of the hemorrhagic septicaemia Pasteurellae occurs readily when both

[4] Friedewald, W. F., and Kidd, J. G., *J. Exp. Med.*, 1940, **72**, 531.

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