Résumé

L'auteur a étudié le nombre de chromosomes dans la spermatogénèse de 11 espèces suivantes du genre *Otiorrhynchus* GERM., provenant de diverses localités en Pologne: *inflatus v. salebrosus* BOH., *multipunctatus* F. (= *irritans* HBST.), *repletus* BOH., *niger* F., *fuscipes* OL., *morio* F., *kollari* GYLL., *equestris* RICHT., *obtusus* BOH. (= *graniventris* MILL.), *corvus* BOH., *obsidianus* BOH. Toutes les espèces étudiées sont bisexuées et diploïdes; le nombre somatique de chromosomes = 22, dont 20 autosomes et 2 hétérochromosomes. La méiose chez toutes ces espèces est tout à fait typique.

A new and simple Method for the Purification and Concentration of Influenza virus

Several methods have been described for the purification and concentration of influenza virus. These methods have recently been summarized by COX *et al.*[1].

The new method described below has proved to be extremely simple and effective.

To 10 ml allantoic fluid containing influenza virus 5 ml of a $Ca(HCO_3)_2$ solution is added, prepared by passing an excess of CO_2 into a saturated solution of $Ca(OH)_2$.

To this mixture 4·5 ml of a saturated $Ca(OH)_2$ solution is added. A precipitate of $CaCO_3$ is formed, adsorbing the virus quantitatively. After centrifuging in an ordinary laboratory centrifuge the clear supernate, showing no virus activity, is discarded. The precipitate is resuspended in a 0·9 per cent NaCl solution, e.g. 5 ml if a twofold virus concentration is desired. In order to bring the p_H up to 7 a small quantity of Na_2CO_3 has to be added to the NaCl solution.

0·5 g sodiumdusarit[2] is added to the suspension and CO_2 is passed slowly into the solution until the precipitate of $CaCO_3$ is dissolved. After centrifuging the supernate proves to contain all the original virus effectively purified, as nitrogen determinations have shown.

The whole procedure can be described in the following scheme (apart from coefficients)

$$Ca(HCO_3)_2 + Ca(OH)_2 + \text{virus} \rightarrow \boxed{CaCO_3} - \text{virus} + H_2O$$

$$\boxed{CaCO_3} - \text{virus} + \boxed{\text{dusarit}} - Na + CO_2 + H_2O \rightarrow NaHCO_3 +$$

$$\text{virus} + \boxed{\text{dusarit}} - Ca$$

According to this method vaccines have been prepared from different virus strains. These vaccines showed excellent antigen properties, the same as the suspension of $CaCO_3$-adsorbed virus. The latter, however, was unstable and lost its agglutination activity within a few days.

Virus recovery was about 100 per cent, and no loss of infectivity as compared with the original allantoic fluid could be observed in egg-infection tests.

In these experiments only the coarse dusarit fraction was used obtained by suspending the commercial product in distilled water and discarding the supernate, which contains the small particles and eventually fine grade impurities. This simple manipulation was repeated until resuspending gave a rapidly sedimenting product and a water-clear supernate. After this the coarse fraction was treated twice with a saturated NaCl solution and finally washed once or twice with distilled water. The product is partially dried at 37° and may be sterilized in a moist atmosphere.

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Zusammenfassung

Es wird eine neue Methode für die Reinigung und Anreicherung von Influenzavirus beschrieben: Das Influenzavirus wird zunächst an $CaCO_3$ adsorbiert. Dieses wird mit Hilfe von eingeleiteter CO_2 in Lösung gebracht. Die Ca-Ionen werden mit Hilfe von Dusarit, einem sehr wirksamen «Permutit»-Körper gegen Na-Ionen ausgetauscht.

Experiments with Nitrogen-Fixing Microorganisms from the Rumen of the Goat[1]

Previous investigations have shown that certain types of the symbiotic microorganisms in the rumen of cow and sheep regularly possess nitrogen fixing capacity[2]. There can be no doubt that this property is common to the whole group of ruminants. It is therefore not surprising that the goat rumen also contains nitrogen fixing bacteria. To test this, Erlenmeyer flasks (300 ml) containing 100 ml of culture liquid[3] were inoculated with 0·05 ml of material taken from the rumen of the goat. An analysis carried out after a period of ten days revealed an increase in the total nitrogen content of about 200%. As nothing is known of the physiological role which the nitrogen-fixing bacteria may play in the economy of their host animal, many questions arise.

First it is desirable to determine the number of the nitrogen fixers and to know whether that number is constant. In an investigation of this kind it is essential to take samples at regular intervals from the contents of the rumen of the same animal. This is possible by means of a permanent rumen fistula, which allows easy access to the rumen, and affords sterile closing when not in use. For the purpose of the experiment a 3–4 year old goat was used[3].

In the winter month, on a daily diet of 1 kg oats + hay + bicalciumphosphate, samples were taken twice monthly. The dilution technique was employed, and on plating out 1 γ of rumen liquid on a nitrogen-free agar medium[2], it was found that 1–9 colonies developed. This reveals the average figure of $5 \cdot 10^6$ nitrogen-fixing microorganisms p. ml.

The figures of the total number of the rumen bacteria, given in the relevant literature, are much higher. The nitrogen-fixing bacteria represent therefore only a small proportion of them. But these figures are scarcely

[1] H. R. COX, J. VAN DER SCHEER, S. AISTON, and E. BOHNEL, J. Immunol. *56*, 149 (1947).

[2] Dusarit, also known as Zeo-Carb, is a sulfonated coal product with powerful permutit properties. It is manufactured by Duper Waterreiniging N. V., Heerengracht 120, Amsterdam. – The efficiency of Dusarit is quite different from that of $Ca_3(PO_4)_2$ as described by J. E. SALK, (Science *101*, 122 [1945]). Dusarit does not consist of $Ca_3(PO_4)_2$, but contains at most an insignificant amount of it as contamination.

[1] I am indebted to the Swedish Wenner-Gren Foundation which has financed this work.

[2] L. TÓTH, Exper. *4*, 395 (1948).

[3] Docent I. SPERBER has kindly supplied me with samples taken from the goat. I thank him for the trouble he has taken in undertaking the surgical procedure.