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.

comparable, since they have been obtained by different counting methods. The average figure of $5 \cdot 10^6$ p. ml represents only the number of rumen inhabitant microorganisms of the goat which are able to grow in the absence of a nitrogen source in the culture medium. This, however, is a specific experimental condition, neither physiological nor optimum, and, as the cultivating procedure by no means resembles the natural condition in the rumen, the correct number of the nitrogen-fixing bacteria of the rumen may be higher.

Change in the diet, from winter fodder to grass, was accompanied by a sudden decrease in the number of the nitrogen fixers, and, it lasted 5–6 weeks, until a new provisional average figure of $2 \cdot 10^5$ p. ml was established.

The microorganisms cultivated from the rumen of the same goat, and on the same nitrogen-free agar medium, reveal colonies of different colour, shape, and size. Microscopical examination has shown that each type of colony contains another bacteria strain. The most common strain forms gelatinous yellowish-coloured colonies of 2 mm in diameter on the surface of the slant agar.

To test the nitrogen-fixing capacity of these bacteria, nitrogen-free culture liquid was inoculated with bacteria suspensions of the different strains[1]. The increase in nitrogen fixed is quite considerable in each case. There results an average value of 10 mg p. l of culture liquid, starting from 2 mg p. l. The differences between the various strains of bacteria from the goat in respect of nitrogen-fixing ability are not so remarkable as, for instance, those in the case of the cow.

As there exist together in the rumen of the same goat several different strains of nitrogen-fixing bacteria, the question arises, whether the nitrogen fixation is not more effective in mixed culture. Three different strains were tested in all seven possible combinations, but no remarkable differences could be observed. However it cannot be concluded that the information derived from this experiment is applicable to the natural condition in the rumen.

The greatest part of the total nitrogen fixed by the cultivated bacteria of the goat rumen, is present as plasma protein of the bacteria. This is separable by Seitz filter. A smaller part of the fixed nitrogen, on the contrary, is able to pass through the sterilizing filtering film. The percentage of the non-bacteria nitrogen is highest on the second day of the experiment (c. 30%) attains its minimum on the 4–5th day and then rises again. This later rising-phase can perhaps be explained by bacteriolysis, but the second day maximum may be due to other causes.

The presence of nitrogen fixing bacteria in the rumen raises the question of the possibility of nitrogen of the air entering the metabolism of ruminants by means of bacterial activity in the rumen. There is now some evidence to disprove this. It is a well-known characteristic of all nitrogen-fixing bacteria that in the presence of bound nitrogen no fixation occurs. The level at which no more fixation takes place is very low, being in the case under consideration below 30 mg p. l of culture medium. Against this the bacteria-free rumen liquid of the same goat shows nitrogen amounts as much as 300 mg p. l or more. It seems therefore improbable that fixation of the molecular nitrogen of the atmosphere can take place in the rumen, at any rate directly and under normal feeding conditions.

L. Tóth

Microbiological Institute, Uppsala-Ultuna, August 27, 1949.

[1] L. Tóth, Exper. *4*, 395 (1948).

Zusammenfassung

In einer mit Pansenflüssigkeit der Ziege beimpften stickstofffreien Nährlösung ist in 10 Tagen eine Stickstoffanreicherung von rund 200% festzustellen.

Aus der Pansenflüssigkeit der Ziege, die durch eine permanente Pansenfistel entnommen wird, lassen sich verschiedene Typen stickstoffbindender Bakterien herauszüchten.

Diese Bakterien vermögen in stickstofffreier Nährlösung Luftstickstoff zu binden. Der Mittelwert der Stickstoffanreicherung beträgt dabei rund 10 mg pro l Nährlösung bei einem Ausgangswert von 2 mg pro l.

Das Ausmaß der Stickstoffanreicherung ist nicht nennenswert verschieden, wenn zur Beimpfung nur ein einziger Typ oder mehrere differente Typen der gezüchteten stickstoffbindenden Bakterien verwendet werden.

Die Zahl der keimungsfähigen Bakterien (auf stickstofffreiem Nährboden) variiert bei demselben Tier und bei gleichem Futter nur in geringem Maße und beträgt bei Winterfutter durchschnittlich $5 \cdot 10^6$ pro ml Panseninhalt. Beim Übergang zu Grünfutter sinkt die Zahl der Bakterien zunächst steil ab und erst nach 5–6 Wochen erreicht sie wieder einen neuen provisorischen Durchschnittswert von $2 \cdot 10^5$ pro ml.

Der gebundene Stickstoff besteht zum größeren Teil aus körpereigenen Stickstoffverbindungen der Bakterien. Den Rest (bis zu 30%) machen wohl andere stickstoffhaltige Verbindungen aus.

Unter normalen Bedingungen dürfte im Pansen keine Fixation von Stickstoff stattfinden, da der Stickstoffgehalt der bakterienfreien Pansenflüssigkeit viel höher ist als die obere Stickstoffgrenze bei der die gezüchteten Bakterien Stickstoff zu binden vermögen.

Antibacterial Activity of Liver Extracts against β-hemolytic Streptococcus

In the course of investigations on the distribution of penicillin in the body, we observed that mashes prepared from normal rat livers have an antibacterial activity against β-hemolytic Streptococcus. The tissue mashes were prepared in (1:1 parts) 1% phosphate buffer p_H 6·0 either by hand grinding with a mortar and pestle, or by mechanical grinding, as described by us elsewhere[1]. The serial dilution technique (Rammelkamp) with β-hemolytic Streptococcus, Strain 4, (Group A, Type 3) as test organism, and rabbit red cells as indicator of hemolysis, was employed. The readings were verified by streaking all tubes on blood agar plates. In fifty experiments liver mashes gave an inhibition of growth up to the fourth serial dilution tube (which corresponded to the inhibition induced by 0·0125 units of penicillin). Expressed in terms of penicillin effect the average inhibition of the liver mashes would be equivalent to 0·22 units of penicillin per gram of liver—which is a relatively high antibacterial activity. That this was a specific inhibition of β-hemolytic Streptococcus by rat liver was demonstrated by (1) failure of identically prepared kidney, lung, spleen, heart, brain, and muscle mashes to show any antibacterial activity, and (2) failure of all organ mashes tested, including liver, to inhibit the growth, when *Bacillus subtilis* (3 R 9675), *Klebsiella pneumoniae* (9997), and *Staphylococcus aureus* (209) were used as test organisms.

[1] B. S. Schwartz and M. N. Lewis, J. Lab. a. Clin. Med. *33*, 904 (1948).