Epidermis, die besonders langsam regeneriert, nicht so ausgeprägt wie in der Norm. Der Effekt der geprüften Substanz scheint sich also in der Hemmung des ganzen regenerativen Funktionszustandes zu äußern, er kann somit eher als *histostatisch* denn als antimitotisch angesprochen werden, im Gegensatz zum Colchicin, das teilungsreife Zellen zur Mitose anregt und sie dann zur Degeneration zwingt und damit ausgesprochen zytoklastisch wirkt[1].

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Anorganisch-chemische Anstalt der Universität Basel und Abteilung für Zoophysiologie des Zoologischen Instituts der Universität Bern, den 26. September 1949.

Summary

The 2-methyl-4-amino-hexanone-(5) (I) which is structurally similar to leucine (II) has a strong inhibiting effect on the regeneration of the tail in *Xenopus* tadpoles. The range of the inhibiting concentration is remarkably wide and the toxicity rather low. The inhibiting effect is not antagonized by leucine. The histological picture of inhibited regenerating tissues suggests a rather histostatic than a pronounced antimitotic action.

[1] M. LÜSCHER, Helv. physiol. acta *4*, 465 (1946).

Cytological Studies upon Genus *Otiorrhynchus (Curculionidae, Coleoptera)* in Poland

The weevil genus *Otiorrhynchus* GERM. is particularly suited to be studied from the point of view of geographical parthenogenesis. There occur parthenogenetic species, and related bisexual ones; among many species of this genus both parthenogenetic and bisexual races are known. In *O. scaber* L. there are even two parthenogenetic races: the one, Alpine, being triploid, and the other, from Finland, tetraploid[1]. From previous research it becomes evident that different races have different geographical range, in some cases the parthenogenetic and bisexual ones coexist in the same localities. It is known, from cytological studies of SUOMALAINEN[2] and SEILER[3] that parthenogenetic races are polyploid in comparison with the bisexual ones, treated as diploid. In genus *Otiorrhynchus* no diploid parthenogenetic race was found, though it occurs in genus *Polydrosus*. As in Finland *P. mollis* STRÖM. breeds in Poland as parthenogenetic and diploid. The occurrence of this race is affirmed in the Pieniny Mountains (a chain of lime rocks north of the Tatras) and near Krakow (not yet published). SUOMALAINEN presumes that parthenogenetic diploid races ought to exist also in genus *Otiorrhynchus*, this being the intermediary stage between bisexual-diploid, and parthenogenetic-polyploid races, as found in *Solenobia triquetrella*[4]. The lack of evidence as to the existence of such a race in genus *Otiorrhynchus* may be due to the scarcity of material studied up to now. SUOMALAINEN expresses his opinion, that geographical parthenogenesis and polyploidy of weevils has a connexion with the glacial period[1]. To prove this, it is necessary to investigate cytologically many species and races, also in different points of their distributional range.

SUOMALAINEN's study covers areas of Finland, Germany, Austria, Switzerland, the results of an analysis of 30 species having been published. SEILER has investigated the cytology of *O. sulcatus* F. occurring near Naumburg. Till recently any knowledge concerning chromosome-relations of weevils in Poland was lacking, although this area, considering its proximity to SUOMALAINEN's area, as well as its specific conditions in the glacial period, deserves attention.

Since 1947 I have collected material for cytological studies of weevils of Poland, especially those of genus *Otiorrhynchus*. One part of my results forms the subject of this notice.

The material described here comes chiefly from the Karpaty Mountains. The 10 species investigated (*inflatus v. salebrosus* BOH., *multipunctatus* F. [= *irritans* HBST.], *niger* F., *fuscipes* OL., *morio* F., *kollari* GYLL., *equestris* RICHT., *obtusus* BOH. [= *graniventris* MILL.], *corvus* BOH., *obsidianus* BOH.) were collected in the Tatra Mountains (Mala Laka Valley, Suchy Zleb-Kalatówki, Gubalówka), in Eastern Beskid (Wielka Roztoka Valley), Western Beskid (Babia Góra), and 1 species, *O. repletus* BOH., endemic for Poland, in the neighbourhood of Warsaw.

The localities cited above have had different conditions during the glacial period. Mala Laka Valley was covered with glacier, the morenes existing till now prove it definitely[1]. From the lower parts of this valley there come very abundantly *O. niger, corvus, multipunctatus*. From the upper parts in neighbourhood of the glacial cirque *O. kollari*. All these species are—in these localities—bisexual. Similar conditions existed in Suchy Zleb where bisexual *O. obtusus* is found. The question of the glaciation of Gubalówka, where *O. fuscipes* taken for investigation were collected, is not yet definitively solved. On the contrary Wielka Roztoka Valley, the habitat of bisexual *O. kollari, niger, equestris, obsidianus, inflatus* was not covered with glaciers. This concerns probably also habitats on the *Babia Góra* slopes where *O. morio* is found.

All (11) species investigated are, in the localities cited above, bisexual and diploid. Chromosome relations of different species are described on the basis of a study of spermatogenesis. Up to this time the course of spermatogenesis was known, according to SUOMALAINEN, in *O. niger, fuscipes, morio, equestris, inflatus*. New results concern *O. corvus, multipunctatus, obsidianus, obtusus, kollari, repletus*.

The diploidal number of chromosomes stated in spermatogonies is 22 (as in the bisexual ones according to SUOMALAINEN), 20 being autosomic and 2 sexual, which differ in size. Meiosis goes on normally. In the first metaphase 11 elements are visible, 10 of which form autosomic tetrades, the 11th being a pair of sexual chromosomes. In the first anaphase the conjugating elements undergo a separation and the plates arising have 11 chromosomes each, 10 of which are autosomes, and the 11th chromosome x or y. The second meiotic division is equational. The further course of spermatogenesis is also normal.

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[1] B. HALICKI, Bull. Serv. Géol. Pologne *5*, 23 (1930).

[1] E. SUOMALAINEN, Sitz.-Ber. Finn. Akad. Wiss. *1944*, 181 (1945).

[2] E. SUOMALAINEN, Ann. Acad. Sci. Fennicae, Ser. A, *14*, 1 (1940); Hereditas *33*, 29 (1947).

[3] J. SEILER, Chromosoma *3*, 88 (1947).

[4] J. SEILER, Rev. suisse zool. *48*, 537 (1941); 3. Jahresber. Schweiz. Ges. Vererbungsforsch. *18*, 691 (1943); Rev. suisse zool. *53*, 529 (1946).

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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Department of Internal Medicine, University Hospital, Leiden, July 20, 1949.

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.