geographic evidence of the hypothesis we have put forward[21].

Leaving open the question of the actual date of the penetration of the ancestors of the subgenera *Citellus* (*s. str.*) and *Colobotis* into the Palearctic, we must accept that the North American longtailed ground squirrels have migrated to Asia across the Bering land bridge during at least two disjunct periods[22].

Выводы *Citellus* (*s. str.*) *relictus*, *C. dauricus* *C. pygmaeus* обладают идентичными кариотипами ($2n = 36$, $NF = 72$). Группа *C.* (*Colobotis*) *erythrogenys* и *C. fulvus* отличается от этих видов лишь незначительными различиями в форме *y*-хромосомы. Кариотип *C.* (*Urocitellus*) *undulatus* ($2n = 32$, $NF = 64$) существенно отличастся от всех описанных выше видов но идентичен кариотипу *C. columbianus*. Обсуждаются причины кариологической гомогенности большинства современных азиатских *Citellus*, характеризующихся аллопатрическим распространением, в сравнении с неарктическими *Citellus*, характеризующимися широким распространением симпатрии и резкими различиями в строении хромосом. Сопоставляются цитологические, зоогеографические и палеонтологические данные по эволюции сусликов.

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[22] We thank Prof. N. I. KALABUKHOV, Prof. N. V. NEKIPELOV and Dr. V. K. SHEPELEVA for some living specimens, Dr. S. I. RAJABLI for the preparation of the chromosomes of *C. dauricus*, *C. pygmaeus* and *C. f. orlovi*; Y. MIROKHANOV, E. IVANITSKAJA and E. ZHOLNEROVSKAJA for their technical assistance.

PRO EXPERIMENTIS

A Nylon Reograd Rotor Embodying New Principles

ANDERSON et al.[1] introduced the principle of the reorienting gradient (reograd) rotor. This rotor was subsequently superceded by the continuous flow and the large scale gradient rotors (as manufactured by Beckman Instruments Incorporated). A detailed review on the application of zonal centrifugation may be found in the Spinco DS326 brochure entitled 'Large-scale Zonal Rotors for use in the Beckman Preparative Ultracentrifuges'.

The reograd rotor was reinvestigated in this laboratory and it was found that after the introduction of a few new features it became a valuable addition to the family of rotors of modern preparative ultracentrifuges.

The Nylon Reograd Rotor. Nylon was selected as a suitable material for the construction of this prototype rotor because of its favourable tensile strength-density ratio, its chemical inertness and the ease with which it may be machined on a lathe.

The rotor described here was machined from a cylindrical block of nylon 21×10 cm. The final product has a diameter near the base of 20.5 cm and 16 cm at the top. The cylindrical cavity which is 12.3 cm wide and 5 cm deep with the base sloping towards the centre at an angle of 3° from the horizontal. The rotor is fitted with a duraluminum lid, and a base of the same material designed

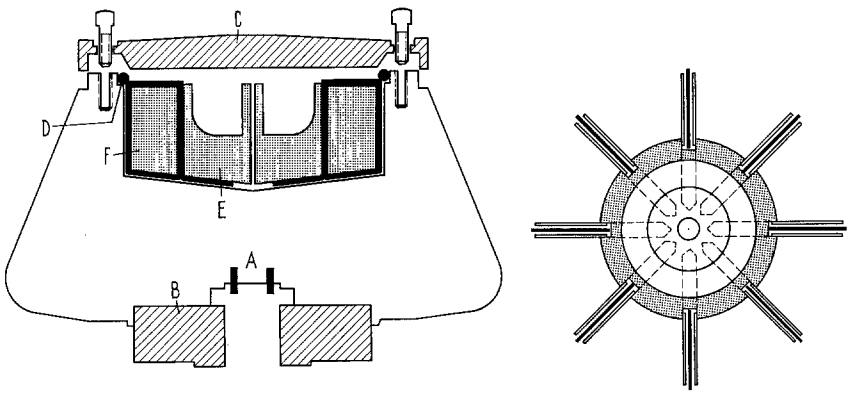

Fig. 1. Left: A cross-sectional drawing of the nylon zonal rotor. A) The main body of the rotor with the cylindrical central cavity. The base of the cylinder slopes towards the centre at 3.0°. B) The base, made of duraluminum, is screwed on to A with 4 steel screws. 2 brass pins protrude from the main body. The base and pins enable it to be driven on the Spinco Model L ultracentrifuge. C) The lid, made of duraluminum is screwed on to the rotor by means of 6 aluminium screws. D) An O ring fits exactly into the rim of the cylinder. When the lid is screwed down a water-tight seal is formed. E) The central core composed of a perspex cylinder into which a cup-like cavity is cut holding an inner column through which a capilliary, 1 mm in diameter, is drilled through its entire length. F) The central core is attached to 8 perspex septa with O rings wrapped over recesses cut into their peripheries. 8 strips of rubber are glued on to the base of the central core to raise it approximately 0.8 mm from the lower surface of the cylinder. Right: A plan view of the central core showing the O rings wrapped around each of the 8 septa, and the rubber strips radiating from the centre. The capillary down the centre rod is also indicated.

to fit the Model L preparative centrifuge. The completed rotor is illustrated in Figure 1, a and b. Further features may be found in the legend to the figures.

Important differences between this and the existing large scale gradient rotors are the wide central core, the increase in the number of speta and the method by which the material for fractionation is introduced on the gradient.

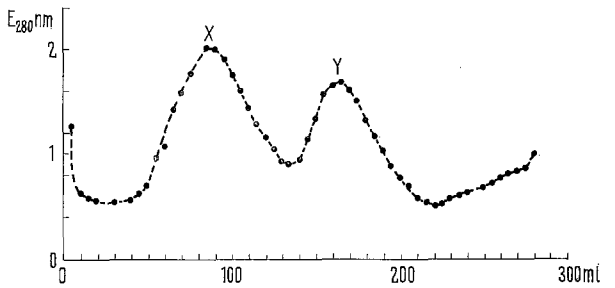

Fig. 2. UV-absorption diagram after centrifugation of the haemocyanin of *Turbo sarmaticus* for 18 h at 12,500 × *g*. Samples for electron microscopy taken at positions indicated *X* and *Y*.

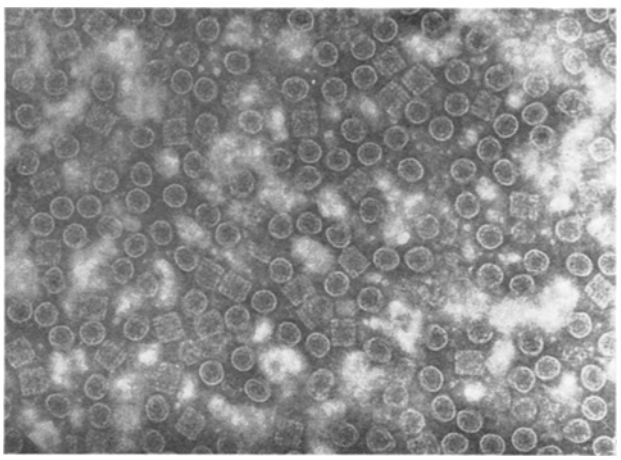

Fig. 3. Electron micrograph of material taken from zone *X* (see Figure 2). Note 'square' particles indicating whole molecules.

Operation. The assembled rotor is placed on a levelling table and the gradient is formed by one of the standard methods. A fine bore truncated needle is lowered through the hole in the central core and the gradient solution of ever-increasing concentration is introduced. In the present work a logarithmic gradient (SVENSON[2]) was used, but a linear concentration gradient would serve the purpose equally well.

It was found that the material to be fractionated could be introduced into the gradient in several ways, but the best way in our experience is to insert the substance to be fractionated in the cup-like cavity and gently accelerating the rotor. During the initial stages the fluid 'slips' on the curved base and moves out further with increasing rotor velocity. Eventually, at still greater velocity, it moves up the vertical wall of the cup to spill over the top through the eight narrow spaces between the lid and the central core onto the gradient surface; 10–20 ml of the sample is optimal.

Application. The use of this rotor is illustrated on the separation of the components of the haemocyanin of *Turbo sarmaticus*. Freshly obtained haemocyanin from this mollusc appeared inhomogeneous. 4 main components having respective sedimentation coefficients 108, 95, 44 and 16 Svedberg units were observed after centrifugation in the Model E analytical ultracentrifuge. In addition to these, small amounts of 2 components of S_{20w} values greater than 108 were observed.

The *T. sarmaticus* haemocyanin was centrifuged in the gradient rotor at 12,500 × *g* for 18 h, the contents syphoned off in 5 ml portions and their UV-absorption determined at 280 nm. The diagram depicted in Figure 2 is obtained. Material collected at positions corresponding to maxima *X* and *Y* was freed of sugar by dialysis against phosphate buffer at pH 7.2 and examined by electron microscopy. The electron micrographs obtained are shown in Figures 3 and 4. From the appearance of the micrographs it would seem that fraction *Y* is constituted of molecules half the size of the whole molecules in fraction *X*.

The separation of the components of a natural mixture of viruses from a partially purified extract of pine

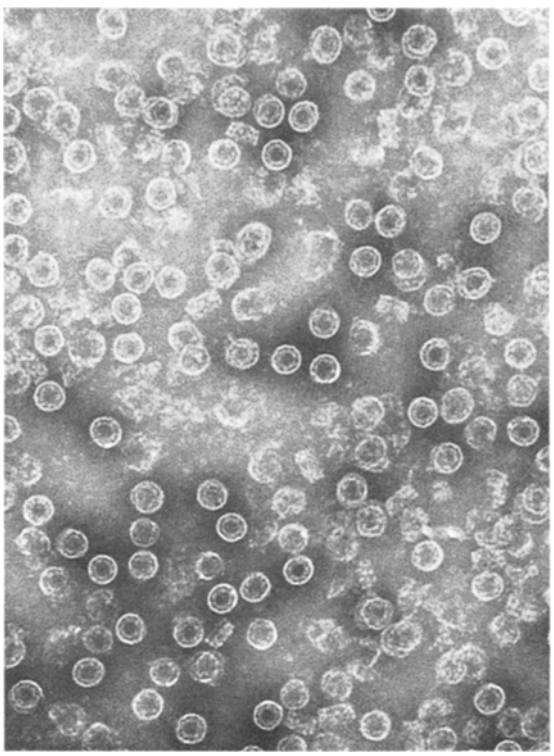

Fig. 4. Electron micrograph of material taken from zone *Y* (see Figure 2). Note absence of 'squares'. Few rectangular particles are seen indicating half molecules.

[1] N. G. ANDERSON, C. A. PRICE, W. D. FISHER, R. E. CANNING and C. L. BURGER, Analyt. Biochem. 7, 1 (1964).

[2] H. SVENSON, *A Laboratory Manual of Analytical Methods of Protein Chemistry* (Eds. P. ALEXANDER and R. J. BLOCH; Pergamon Press, London, Oxford, New York, Paris 1960), vol. 1, p. 216.

emperor moths *Nudaurelia cythera capensis* was attempted. Electron microscopic studies on material taken from the 3 zones observed revealed that there were 3 infective agents present. The largest of particle size 39 nm sedimented out furthest to be followed by a virus type of particle size 37.5 nm. This type represented approximately 80% of the total virus present. This fraction was then followed by a type of particle size 27 nm which represented approximately 5% of the total virus concentration. The entity of particle size 37.5 nm was approximate 100% pure, but the remaining 2 were contaminated with the 37.5 nm variety. The presence of these viruses in the pine emperor moths had been reported on by JUCKES[3].

In conclusion it may be stated that the method could be complementory to such techniques as are commonly used in virus research such as ion exchanging, gel exclusion chromatography and zone electrophoresis.

Zusammenfassung. Der verbesserte, neu konzipierte Reograd-Rotor eignet sich für die präparative Zentrifugierung von Makromolekülen und Viren.

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[3] I. JUCKES, to be published in the Bull. S.A. Soc. Plant Path. Microbiol. (1970).

[4] Acknowledgements. The authors wish to acknowledge the donation of the insect virus concentrate by Mr. I. JUCKES and to Prof. A. KIPPS for the haemocyanin and for his interest in this work. We are grateful to the Department of Agriculture Technical Services for financial assistance towards this investigation.

Automatische Apparatur zur kontinuierlichen Herstellung synchroner Algenkulturen

Zur Durchführung gewisser biochemischer Untersuchungen benötigen wir grössere Mengen synchroner *Chlamydomonas*-Zellen. Um die zeitraubende und umständliche chargenweise Kultivierung zu umgehen, haben wir eine Anlage gebaut, welche möglichst weitgehend automatisiert ist und sich dennoch für verschiedene Zwecke einsetzen lässt. Kontinuierlich arbeitende Apparaturen sind schon verschiedentlich entwickelt worden[1,2] und auch schon im Handel erhältlich (zum Beispiel Chemap AG., Männedorf/Zürich, oder New Brunswick Scientific Company, New Jersey). Anlagen zur kontinuierlichen Synchronkultur dagegen sind noch selten beschrieben worden[3–5]. Unter kontinuierlich verstehen wir hier eine sich über längere Zeit synchron entwickelnde Kultur, welche periodisch verdünnt wird. Wir haben uns zu einer eigenen Konstruktion entschlossen, weil uns die Fragen der Beleuchtung, der Sterilisierbarkeit und der Verfolgung und Steuerung des Wachstums für unsere Zwecke noch nicht befriedigend gelöst erschienen.

Apparatur. Es wurden zwei identische Apparaturen aufgebaut, welche einerseits aus dem eigentlichen Kulturgefäss und andererseits aus den Steuerorganen bestehen.

Kulturgefäss. Das Kulturgefäss, dessen Konzeption auf einer Idee von K. ERISMANN (Pflanzenphysiologisches Institut Bern) beruht, wird durch drei koaxiale Glaszylinder von je 1 m Länge und von 160, 88 beziehungsweise 56 mm Aussendurchmesser gebildet (Figur 1), welche in den Rillen zweier Flansche aus rostfreiem Stahl laufen. Gummiringe und Zugstäbe, welche die Flansche gegeneinanderziehen, sorgen für die Dichtigkeit der abgetrennten Räume. Beide Flansche sind in der Mitte durchbohrt, so dass eine 100 W Hochleistungs-Leuchtstoffröhre das Gefäss von innen her beleuchten kann. Der Raum a) dient, mit Zu- und Abflüssen in beiden Flanschen versehen, der Thermostatisierung durch einen Wasserkreislauf. Der Raum b) nimmt etwa 10 l Nährlösung (NL) auf; eine Teflon-Siebplatte c) im untern Flansch erlaubt Begasung und gleichzeitig Durchmischung der Suspension; das Gasgemisch verlässt das Kulturgefäss zwangsläufig durch ein im obern Flansch verschiebbar befestigtes Glasrohr d), welches in eine Kugel mit zwei Austrittsöffnungen mündet. In dieser Kugel findet beim Nachströmen frischer NL Separation statt: das Gas gelangt durch die obere Öffnung durch ein Sterilfilter ins Freie und die Flüssigkeit strömt durch die untere in ein Auffanggefäss. Ein Widerstandselement in einem Tauchrohr e) misst die Temperatur der Kulturflüssigkeit. Die Suspension wird ausserdem am untern Flansch dauernd entnommen und durch einen externen Kreislauf durch den obern Flansch wiederum in den Kulturraum zurückgeleitet. Dieser Kreislauf wird durch eine eigens für diesen Zweck konstruierte, autoklavierbare Zentrifugalpumpe aufrechterhalten; die Pumpe besteht aus Teflon und kann,

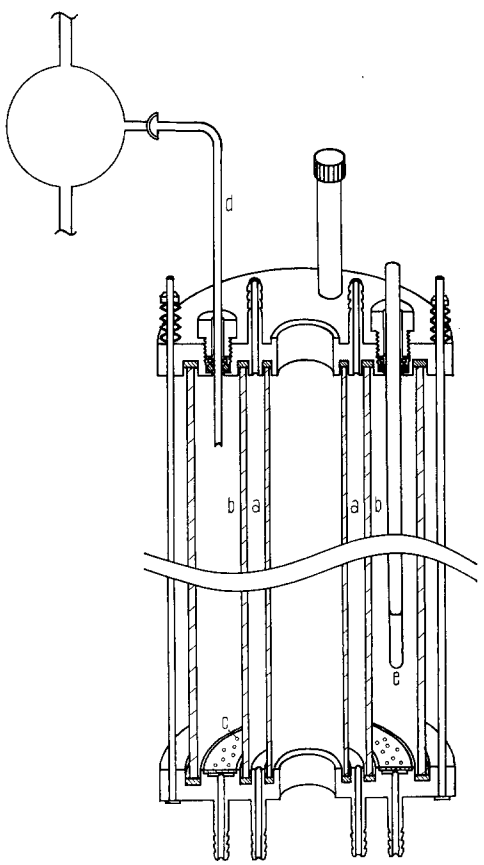

Fig. 1. Kulturgefäss.