pyocyaneum, *B. prodigiosus*. *Coli* UQ, *Coli* SG, and *Coli* IO remained unchanged after treatment with the nucleoproteins of all the other strains.

(B) *Proteus* OX 19: this strain, which does not attack lactose, fructose, and xylose, was treated with the nucleoprotein fraction isolated from *Proteus* OX 2. *Proteus* OX 2 cannot oxidize lactose, but can easily oxidize xylose and fructose. After 6 passages with the nucleoprotein from *Proteus* OX 2, *Proteus* OX 19 has developed the enzymes necessary for the oxidation of fructose and xylose, but did not attack lactose.

Treatment of *Proteus* OX 19 with the nucleoprotein fraction of bacteria of different species has shown that this strain also can mutate with the nucleic acids from some microorganisms of different, but biologically related, species. I have observed mutation in the oxidation of all three substrates mentioned above after treatment with the nucleoprotein from *Coli* I (13th passage). I have also observed mutation in the oxidation of lactose and fructose by the nucleoprotein fraction isolated from *Coli* SG. No mutation was observed with the nucleoprotein from *Coli* IO and *Coli* UQ. After treatment with the nucleoprotein of *S. typhi murium* and *S. newport*, which oxidize xylose and fructose, but cannot attack lactose, *Proteus* OX 19 has attacked xylose and fructose, but not lactose.

The mutation has failed with the nucleoprotein fraction of *Staphylococcus* Oxford, *B. subtilis*, *B. pyocyaneum*, and *B. prodigiosus*.

The transforming principle is resistant to tryptic and peptic digestion, and to ribonuclease, but is completely destroyed by desoxyribonuclease. These experiments confirm BOIVIN's results. Partial mutation in the enzymatic equipment has been observed in *Coli* I and in *Proteus* OX 19 also after treatment with desoxyribonucleic acid isolated from bacteria of different, but biologically related species, e. g. other Enterobacteria.

The experiments reported here give experimental support to KAUFFMANN's observation who isolated from fæcal matter of normal subjects and of patients with gastroenteric diseases strains of *B. coli* containing some antigenic fractions of *Salmonellæ*. Explanation is given also to the paraagglutination phenomenon described by ZIRONI. It follows that the presence of common antigenic fractions in bacteria is likely to be of mutative origin.

MARIO UMBERTO DIANZANI

Department of General Pathology, University of Siena, November 14, 1949.

Résumé

L'auteur a obtenu des mutations de l'équipement enzymatique (oxydation de la saccharose) d'une souche de *E. coli* traitée avec la fraction nucléoprotéique extraite de deux souches diverses du Colibacille attaquant ce sucre. Une souche de *Proteus* OX 19 également a muté par l'oxydation des deux sucres, xylose et fructose, sous l'influence d'un principe de nature nucléoprotéique extrait d'une souche de *Proteus* OX 2 attaquant ces substances. D'autres mutations ont pu être obtenues par l'oxydation des carbohydrates dans les mêmes souches de Colibacille et de *Proteus*, à la suite du traitement avec la fraction nucléoprotéique extraite de quelques souches de bactéries intéstinales appartenant à des espèces différentes (*Coli*, *Proteus*, *Salmonella*), mais biologiquement parentes. L'auteur n'a pas obtenu de mutations chez le Colibacille et le *Proteus* en se servant de nucléoprotéines extraites de souches biologiquement éloignées. Le principe actif peut être identifié avec l'acide désoxyribonucléique, car il reste inaltéré après la digestion tryptique et la digestion peptique et résiste à la ribonucléase, tandis qu'il est complètement inactivé par la désoxyribonucléase.

Experiments with Chromosomes Isolated from Intermitotic Nuclei

By manipulating his preparation of *chromosin*—a desoxyribosenucleoprotein complex derived from the cell nucleus—from liver, kidney, and other tissues, MIRSKY[1] found certain bodies which he explained as isolated chromosomes. The *chromatin threads* isolated from the intermitotic (resting) nucleus vary little in size and organization. Usually they show distinct doubleness, a specific pattern with satellites, heterochromatic and euchromatic sections, and often one finds a repeated occurrence of a precise type of chromosome. As with chromosomes, the threads can be uncoiled by agents such as KCN. Some bodies considered as nucleoli can be seen in a smear, stained with FEULGEN's reagent and light green, still attached to a chromosome or as separate particles. Likewise the *chemical* composition of the isolated chromatin threads has already been described by MIRSKY[2]. Because CLAUDE and POTTER[3] isolated chromatin threads which they suggested as represented chromosomes, I regarded it as desirable to examine MIRSKY's bodies by means of polarized light and in regard to their microdissectional behaviour.

In addition to cultures of muscle explants[4] grown *in vitro* after aseptic extraction from *Calliphora erythrocephala* MEIG., I explanted similarly the *follicle epithelium* of Drosophila spec., and then I fragmented the cultures *in vitro*, suspended in a large volume of saline, by placing them in a colloid mill. By such *treatment*, all the cells and nearly all the nuclei are broken, and by centrifuging a bright-coloured precipitate under a dark-coloured supernatant is obtained. In order to reduce autolytic changes, the procedures have to be carried out as rapidly as possible, and must be performed in a cold room kept at 1° C. Rise of temperature, for the same reason, has to be prevented. After suspension in a saline, the precipitate is placed in the colloid mill once more for 1–2 minutes. After continued washing and centrifuging the precipitate consists, at last, of chromosome threads isolated from intermitotic nuclei of the explant.

Microdissectional stretchings between microneedles have been made as usual. The *measurements of birefringence* (path difference or retardation, to be divided by the length of the path travelled by light) have been performed with a BRACE compensator (rotating λ/30-mica compensator) and the half-shadow wedge according to J. MACÉ DE LÉPINAY[5]. Measurements with this method are known to be extraordinarily sensitive[6].

The *isolated threads* present, to say the least, a model of a chromosome, being an aggregate of polypeptide protamine molecules in association with nucleic acid. Often they possess two arms separated by a (primary) constriction which cannot be stained by FEULGEN's reagent. In addition to that, one often sees small secondary constrictions. *Between crossed nicols* one observes, in general, no reliable indication of birefringence. But if the bodies are treated with ethyl

[1] A.E. MIRSKY and A.W. POLLISTER, J. Gen. Physiol. *30*, 117 (1946), with H. RIS, *ib.* *31*, 1 (1947).

[2] A.E. MIRSKY and H. RIS, J. Gen. Physiol. *31*, 7 (1947).

[3] A. CLAUDE and J.S. POTTER, J. Exp. Med. *77*, 345 (1943).

[4] H.H. PFEIFFER, Z. Naturforsch. *4b*, 307 (1949).

[5] H.H. PFEIFFER, *Das Polarisationsmikroskop als Meßinstrument in Biologie und Medizin* (Friedr. Vieweg & Sohn, Braunschweig, 1949), S. 55, 64.

[6] H.H. PFEIFFER, Optik (Stuttgart) *5*, 217 (1949).

alcohol[1] or stretched between microdissection needles[2] there is found double refraction of slight intensity ($\Delta n = -0{\cdot}0004$ till $0{\cdot}0019$). Its character (sign) is negative with respect to the length of the object. Hence it follows that nucleoprotein is present. After previous treatment with acetic acid vapour one finds a weak swelling of the bodies and in some cases a positive double refraction. By digesting the nucleic acid of the chromatin threads by means of milt nuclease[3] it is possible to prevent a disturbance of the leptonic structure. Then the stainability according to FEULGEN disappears, but the ninhydrine test turns out positively over the entire length of the threads. In addition to this, one observes a decrease of the negative birefringence, often until isotropy, or, not rarely, over the whole length of the threads until a very weak positive birefringence appears. Experimental stretching of the threads establishes that the intensity of positive double refraction is largely due to the different stretching rates, but perhaps also to a certain extent to the increased disordering action of the BROWNian movement. Investigations treating the isolated threads as WIENER Mischkörper (composite bodies, the double refraction of which may be distinguished as textural and intrinsic birefringence) are yet in process, but some results in regard to this have been obtained by means of histological imbedding and subjection to the alcohol series.

The *nucleic acid molecules* in the protein fibres of the threads do not show a complete *orientation*. Compared with artificial fibres of sodium thymonucleate, on the contrary, the nucleic acid particles within the Mirsky bodies yield only a small birefringent effect. We assume therefore, that the nucleic acid chains are intercalated with a delimited orientation rate[4]. Provisional calculations by means of FREY-WYSSLING's method[5] of the scattering angle verify this conclusion. On the assumption of a high scattering of the nucleic acid chains in the threads, the weak birefringent effect might be understood as a tendency toward orientation that resulted in the shrinkage or swelling of the preparation. Nevertheless, we assume the *protein chain structure* to be continuous, for the orientation of the polypeptide chains causes the anucleal chromosome segments to appear positive birefringent. Aside from the lack of full evidence in favour of such an assumption, I think that the protein component of the threads might appear as a linear sequence of highly organized segments, perhaps more closely analogous to a particle of tobacco mosaic virus than to a thread of myosin[6]. Such considerations open more prospects, I believe, than explanations formerly attempted with the aid of the spiral structure[7].

If we survey all the investigations as a whole we are inclined to consider the chromatin threads isolated by MIRSKY as being *true chromosomes of intermitotic nuclei*[1]. It would be most opportune now to examine the isolated threads by means of the electron microscope.

HANS H. PFEIFFER

Laboratory for Polar.-Microscopy, Bremen, February 15, 1950.

Zusammenfassung

Aus intermitotischen Kernen in Gewebekulturen wurden isolierte Chromatinfäden gewonnen. Sie wurden mikrochirurgisch und zwischen gekreuzten Nicols untersucht. Die erhaltenen Körper sind wahrscheinlich richtige Chromosomen. Das geht nicht nur aus ihrer morphologischen Beschaffenheit und chemischen Zusammensetzung hervor, sondern auch aus ihrem Verhalten im polarisierten Licht.

[1] W. A. BECKER and I. KOBZIAL, Act. Soc. bot. Polon. *14*, 239 (1937).

[2] H. H. PFEIFFER, Chromosoma *1*, 526 (1940); *2*, 77 (1941).

[3] D. MAZIA and H. JAEGER, Proc. Nat. Acad. Washington *25*, 456 (1939).

[4] H. H. PFEIFFER, Nature *162*, 419 (1948).

[5] A. FREY-WYSSLING, Chromosoma *2*, 473 (1943). – H. H. PFEIFFER, Z. Naturforsch. *1*, 461 (1946).

[6] J. B. BATEMAN in: RUD. HÖBER, *Physical Chemistry of Cells and Tissues* (The Blakiston Co., Philadelphia and Toronto, 1945) p. 93, 186.

[7] Y. KUWADA and T. NAKAMURA, Cytologia *6*, 78 (1934). – Y. KUWADA, Fujii Jubil. Vol. (Maruzen Co., Tokyo, 1937) p. 482. – But cf. also: A. FREY-WYSSLING, *Submicroscopic Morphology of Protoplasm and its Derivatives* (Elsevier Co., New York and Amsterdam, 1948) p. 147.

[1] Cf. M. J. D. WHITE, *The Chromosomes* (Methuen, London, 1937) p. 11. – C. H. WADDINGTON, *An Introduction to Modern Genetics* (Allen & Unwin, London, 1939) p. 377. – J. B. BATEMAN, l. c. 184. – H. H. PFEIFFER, *Submikroskopische Morphologie des Zellkerns und der Chromosomen*, in: A. JORES, *Synopsis* (Claassen & Goverts, Hamburg, in the press).

Polyploïdie intraspécifique chez *Cerastium arvense* L. et nombres chromosomiques de quelques autres *Cerastium*

Au cours des recherches cytologiques que nous poursuivons sur le genre Cerastium, nous nous sommes particulièrement intéressé au *C. arvense* L. dont le polymorphisme est bien connu.

Le matériel étudié comprend pour la plupart des plantes récoltées dans la nature et cultivées au jardin botanique de Neuchâtel.

Les numérations chromosomiques ont été faites en général à la méiose, mais parfois aussi sur les méristèmes radiculaires. Les résultats de notre étude sont consignés dans le tableau.

Le C. *arvense* n'a été étudié jusqu'ici au point de vue caryologique que par ROHWEDER[1] et par Madame R. MATTICK-EHRENSBERGER. Les résultats de cette dernière, non encore publiés, nous ont été communiqués par lettre. Ces deux auteurs ont trouvé $2n = 72$. La plante étudiée par ROHWEDER appartenait à la ssp. commune.

Il nous a donc été possible de découvrir dans cette espèce linnéenne 2 races chromosomiques différentes, l'une à $n = 18$ que nous appellerons diploïde, l'autre à $n = 36$ que nous appellerons tétraploïde. En effet, aucun *C.* à $n = 9$ n'a encore été découvert.

Au point de vue systématique, il est intéressant de constater que la ssp. commune, répandue en plaine n'a pas le même nombre chromosomique que la ssp. strictum qui habite les Alpes. Les anciens auteurs (par exemple LAMARCK et DE CANDOLLE) considéraient cette dernière comme une bonne espèce. Notre étude cytologique montre clairement que la ssp. strictum n'est pas un simple accommodat, une forme des hautes altitudes du *C. arvense*.

Les essais de culture nous ont conduit au même résultat, particulièrement en ce qui concerne les plantes 6 et 7. Des plantes élevées de graines récoltées au Col du Jorat ont gardé leur port particulier. Ces observations s'expliquent fort bien par la différence du caryotype. La ssp. suffruticosum, par contre, diffère très peu de la ssp.

[1] Beih. Bot. Centralbl. *59*, 1 (1939).