washed with H_2O, the lysates diluted 10-fold (10% final urea conc.) with the rinsing-water[2] as soon as possible and stored at 4°C. The whole procedure involved about 60 min. For inoculation, a 10^{-3} dilution with bovine amnion fluid was made and assayed as described. Control flasks received similarly diluted UDC. The TC exposed to lysates were destroyed within two days, whereas controls treated with urea-desoxycholate alone remained intact, permitting growth and subcultivation of the cells. It seemed that the lysates were carrying (among others) *biologically active virus material* set free by UDC and preserved by the timely dilution[2]. This assumption was substantiated by the following experiments. *The cell residues of positively infected and destroyed (CPE-positive) TC* were dissolved in UDC as usual and their preserved infectivity demonstrated. HeLa cells inoculated with this lysate exhibited visible CPE after 16 h and the whole culture was destroyed within two days. Finally if 10% Urea-lysates were added to the TC and after 5 min diluted 3 times with amnion fluid, immediate destruction of the cells by the UDC occurred. This was proved with the large 'total' pool illustrated in the Table (group 4) where medium and cells were lysed together. The tenfold diluted lysates, however, do not retain intactly their infectivity during 7 weeks storage at 4°C; if tested in 10^{-3} dilution, CPE was not apparent only after 6 days, when overnight total destruction of the inoculated TC occurred. Second passages of the cultures destroyed were positive, confirming the presence and successful transmission of infective material with homogenates and lysates (Table). Concentration, purification, and isolation of the active principle is under study[4].

We might summarize that the above findings were repeatedly observed and may prove the following assumptions; (1) The CPE-negativity of unsuccessfully infected TC may depend on a peculliar behaviour of the cells against polio virus (2). The infective particle seems to be firmly associated to certain cells without apparent cytopathogenic effect, and can be liberated by *physical* (thermal-shock, grinding) and/or *chemical* means (3). UDC-treatment *did not destroy the biological activity* of the virus examined (4). The UDC reagent seems to be the simplest *chemical* tool[5] for the liberation of infectious material from true or latent poliomyelitis infection *in vitro*, although the nature of this procedure has to be clarified.

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Zusammenfassung

Stark cytolytisch wirkende Urea-Desoxycholatlösung zertört die biologische Aktivität des Poliovirus (Typ I, Mahoney-Stamm) *nicht;* somit ist dieses Mittel zur Freisetzung infektiösen Materials aus latent oder manifest infizierten Zellkulturen sehr geeignet.

[4] In preparation.

[5] J. S. COLTER, *Nucleic Acid as Carrier of Viral Activity* in E. BERGER and J. L. MELNICK, *Progress in Medical Virology*, vol. I (S. Karger, New York–Basel 1958), p. 1.

Metabolic Interactions In Vitro between Polymorphonuclear Leukocytes and Pathogenic and Nonpathogenic Microorganisms[1]

The addition of bacteria to polymorphonuclear leukocytes in the Warburg respirometer causes an alteration of the otherwise constant rate of oxygen uptake by the leukocytes. Changes of rates may be attributed to the effect exerted by bacteria on the leukocytes and by leukocytes on the phagocytized bacteria[2–4]. In previous studies it had been shown that tubercle bacilli maintained a constant rate of respiration in an intracellular environment as evidence of their ability to survive within phagocytic cells[5]. These investigations were extended to other pathogenic and nonpathogenic bacteria. The results of these studies are reported.

Methods. Bacteria were grown in brain-heart-infusion broth and were washed repeatedly. Peritoneal exudate leukocytes from rabbits were obtained by injecting a solution of sodium caseinate intraperitoneally 15 h prior to collection. The cells were washed twice in saline; leukocytes and bacteria were resuspended in a medium consisting of Krebs Ringer phosphate buffer containing 20% homologous, heat-inactivated serum, and 100 mg% glucose. The Warburg vessels were prepared as follows: Leukocyte suspension in main chamber, bacterial suspension (live or heat-killed bacteria) in side-arm, and 10% NaOH in center well. After equilibration in the water bath, the bacteria were added to the leukocytes by tipping the content of the side-arm into the main chamber. Manometric readings were taken every 30 min over a period of 3 to 4 h. In every experiment leukocytes alone, bacteria alone, and leukocytes with live and dead bacteria were used. Samples of mixtures and bacterial suspensions were removed from the flasks to determine number of viable bacteria and extent of phagocytosis.

Results. When oxygen uptake of leukocytes alone with that of leukocytes with *heat killed* bacteria was compared, the results represented in Table I were obtained. The average number of leukocytes per flask was 1×10^8, and there were about 2 to 5 times as many heat killed bacteria. By dividing the rate of oxygen consumption of phagocytes with bacteria by the rate of consumption by phagocytes alone, a ratio is obtained which indicates the difference between the two sets of flasks. As can be seen, the leukocytes showed increased respiration (up to 50%) after having ingested heat killed, *nonpathogenic* bacteria, whereas a reduction of oxygen consumption occurred when the leukocytes had phagocytized heat killed, *pathogenic* organisms.

The respiration of living intracellular bacteria was calculated indirectly, assuming identical oxygen consumption by phagocytes regardless of whether or not the ingested organisms were living or dead. Including the necessary control flasks, one could obtain values for oxygen consumption by bacteria within phagocytes and by bacteria suspended in cell free media. The ratios of these two

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Table I

Effect of Phagocytosis of Heat Killed Pathogenic and Nonpathogenic Microorganisms upon Oxygen Consumption of Polymorhponuclear Leukocytes

Organism	Pathogenicity	Ratio of respiration* $\frac{\text{leukocytes + bacteria}}{\text{leukocytes alone}}$
Staphylococcus epidermidis, Coagulase –	nonpathogenic	1·47 ± 0·12
Pneumococcus II, rough	nonpathogenic	1·35 ± 0·1
Pneumococcus II, smooth in presence of antibody	pathogenic	1·33 ± 0·53
Staphylococcus, Coagulase +	pathogenic	0·78 ± 0·29
Salmonella typhosa	pathogenic	0·98 ± 0.02
Neisseria gonorrhoeae	pathogenic (not for rabbit)	0·81 ± 0·21

* Oxygen uptake in µl/h.

values are given in Table II for several species of bacteria. The respiration of *nonpathogenic* bacteria decreased rapidly upon phagocytosis, and bacterial plate counts revealed a decrease in the number of viable units in the mixture, although stainable intracellular organisms remained visible throughout the observation period. According to plate counts, *virulent* organisms remained viable within phagocytes for the entire observation period. However, three different patterns of intracellular respiratory activity were observed: (1) Intracellular respiration was extremely low with *Neisseria gonorrhoeae*. (2) Intracellular respiration was reduced up to 50% with a non-hemolytic but coagulase positive *Staphylococcus aureus* and a smooth, type II pneumococcus. (3) Respiration was increased when *Salmonella typhosa* and a hemolytic, coagulase positive *Staphylococcus aureus* were phagocytized.

Discussion. Stimulation of respiration of phagocytes during and shortly after phagocytosis has been described to occur with *Brucella* and *Mycobacterium*[3,4]. The extent of stimulation was similar to that found in our experiments (Table I). However, in the case of nonpathogenic, heat killed organisms this stimulatory effect persisted over a prolonged period of time indicating that possibly some of the bacterial materials were available as substrate for the leukocytes. The lack of increase or even the reduction of respiration found when dead pathogenic microorganisms were phagocytized, is surprising. It is unlikely that exotoxins are responsible for this inhibition, since the organisms had been washed repeatedly and heated. The presence of endotoxin-like substances and their intracellular release is more likely, especially with *Neisseria* and *Salmonella*. The increased rate of respiration during phagocytosis of heat killed pathogenic pneumococci in presence of antibody had been observed earlier and was found to be proportional to the amount of antibody on the surface of bacteria[2,6]. This antibody effect can best be explained by a recent finding, that leukocytes ingest and degrade soluble antigens when combined with specific antibody[7]. Nonpathogenic organisms, as expected, ceased to respire after ingestion by phagocytes. This is also true for the virulent pneumococcus, since being an obligate extracellular parasite, it is destroyed fairly rapidly after phagocytosis[8]. Pathogenic organisms continue to respire even after having been phagocytized, some do so at an increased rate (Table II). This finding is compatible with the fact that some of these organisms, depending on their pathogenicity, are known to survive or multiply in the intracellular environment[9]. The failure of *N. gonorrhoeae* to consume oxygen within rabbit leukocytes can be ascribed to its lack of pathogenicity for the rabbit. It is interesting to note, that one of the coagulase positive staphylococci behaved like a nonpathogenic organism, whereas the other reacted as a pathogenic one. This observation that two strains of *Staphylococcus*, indistinguishable on the basis of the coagulase test, behave differently in our experiments might help in explaining some conflicting reports in regard to the ability of staphylococci to survive phagocytosis[10,11].

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Table II

Oxygen Uptake of Pathogenic and Nonpathogenic Microorganisms within Leukocytes Compared with Freely Suspended Microorganisms

Organism	Pathogenicity	Ratio of respiration* $\frac{\text{bacteria within leukocytes}}{\text{bacteria alone}}$
Staphylococcus epidermidis, Coagulase –	nonpathogenic	0·18 ± 0.12
Pneumococcus II, rough	nonpathogenic	0·085 ± 0·12
Staphylococcus, Coagulase + *non* hemolytic	pathogenic	0·53 ± 0.07
Pneumococcus II, smooth in presence of antibody	pathogenic	0·79 ± 0·08
Salmonella typhosa	pathogenic	1·19 ± 0.05
Staphylococcus, Coagulase + *hemoiytic*	highly pathogenic	1·5 ± 0·14
Neisseria gonorrhoeae	pathogenic	0·18 ± 0·03

* Oxygen uptake in µl/h.

The results indicate that intracellular respiration can serve as an indicator of the outcome of the interaction between phagocytes and microorganisms and might allow rapid differentiation between obligate extracellular and facultative intracellular microorganisms[12].

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Zusammenfassung

1. Es wird gezeigt, dass der Effekt von phagozytierten, *hitzeabgetöteten* Bakterien auf die Atmung von Kaninchen-Leukozyten *in vitro* von der Virulenz der verwendeten Mikroorganismen abhängig ist.

2. Die Tatsache, dass *lebende* Mikroorganismen nach Phagozytose intrazellulär fortfahren zu atmen, wird demonstriert und deren Beziehung zur Pathogenität der Bakterien diskutiert.

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[13] Present Address: University Childrens Hospital, Zurich (Switzerland).

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Karyotype in two Himalayan species of Polygonatum

The liliaceous genus *Polygonatum* is believed to have its centre of diversification in Eastern Himalayas and Western China. The present Himalayan species *P. verticillatum* Allioni and *P. cirrifolium* Royle, belong to the group Verticillata Baker. The former species has a wide geographic distribution; besides occurring throughout the Himalayan range, it is extended as far as Northern Europe. Extensive cytological investigations have been carried out on eighteen European strains of *P. verticillatum* and the occurrence of diploid and polyploid forms were reported[1,2]. Though the chromosome number of all the different diploid forms studied[1,2] was the same ($2n = 28$) differences in karyotype were observed from strain to strain, thus revealing a marked intraspecific structural heterozygosity. The karyotype reported[1,2] for the group Verticillata is characterised by the presence of long chromosomes with subterminal primary constrictions and short chromosomes with subterminal and median primary constrictions. The secondary constrictions are present only in long chromosomes.

In the present study a number of strains of *P. verticillatum* and *P. cirrifolium* from Western Himalayas were analysed. The chromosome numbers, $2n = 30$, 64 in *P. verticillatum* and $2n = 38$ in *P. cirrifolium* were determined from root tip somatic plates and the division of the generative nucleus in the pollen tube (Figures 1, 2, 3). The chromosome complement of *P. verticillatum* can be broadly classified into three groups, (i) one pair of long chromosomes with nearly median primary constrictions, (ii) six pairs of medium chromosomes with subterminal primary constrictions, and (iii) eight pairs of short chromosomes with subterminal and median primary constrictions. The secondary constrictions were revealed in two pairs of long and short chromosomes. The karyotype varies in different strains studied and structural heterozygosity in the chromosome complement was also noticed. Thus the present species differs from its European forms not only in the chromosome numbers ($2n = 30$) but also in gross chromosome types i.e. the presence of an additional pair of long chromosome with nearly median primary constriction and of a pair of short chromosome with secondary constriction (Fig. 1). Such a chromosome complement has been reported[1] to be typical of group Alternifolia, Baker, and so far has been unknown for the group Verticillata.

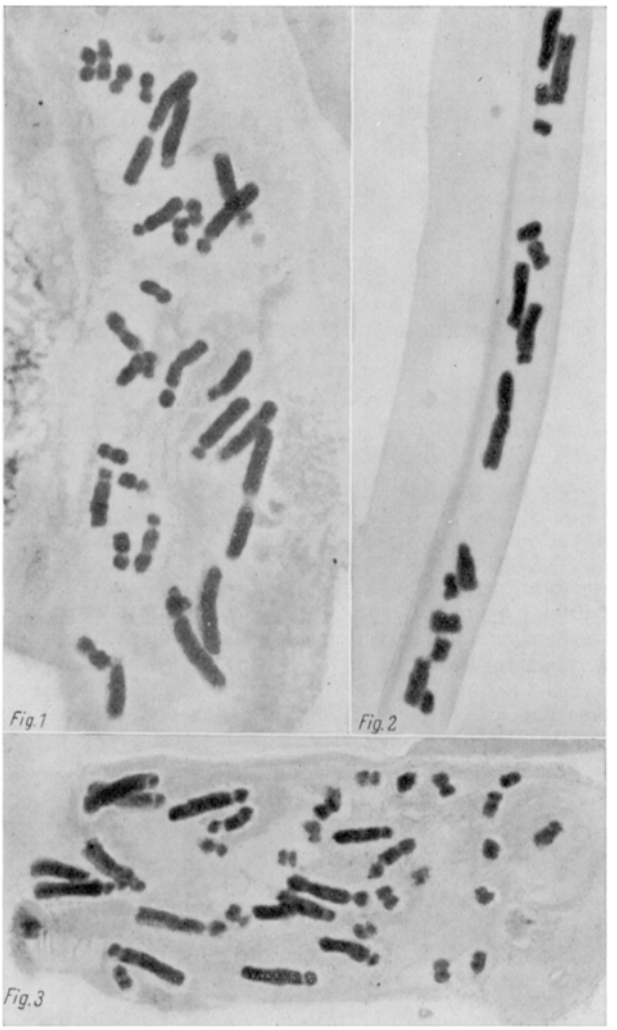

Fig. 1. – Mitotic Stage in Root Tip Cell showing 30 Chromosomes.
Fig. 2. – The Division of the Generative nucleus in the Pollen Tube showing Haploid Chrom. Complement.
Fig. 3. – *P. cirrifolium:* Root Tip, Mitotic Stage showing 38 Chroms.

The karyotype of *P. cirrifolium* ($2n = 38$) is found to be allied to 'Verticillata' type[2] of chromosome complement as it lacks the long chromosome with sub-median primary constriction. However, the number of long and medium chromosomes (seven pairs) is more or less constant and also two pairs of long and short chromosomes have secondary constrictions. In this respect it is similar to the Himalayan forms of *P. verticillatum*. The karyotype of *P. cirrifolium* differs from *P. verticillatum* by the presence of four additional pairs of short chromosomes in the former.

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