phosphorus was estimated in terms of P_2O_5 by the vanadomolybdate method[6] in a Klett photoelectric colorimeter using 420 mμ filter. The results (Table I) showed that significant inhibition of phosphate-dissolving activity had occurred with regard to two of the four bacteria tested in the present study. However, interestingly enough, it was found that *Cephalosporium* sp. alone (in control series) had released as much phosphorus as the individual bacteria did. Some soil fungi are known to solubilize phosphates[7]. Nevertheless, it was considered important to extend the study to determine the phosphate-dissolving ability of the fungi occurring on the surface of nodules. The results of such a study (Table II) confirmed not only the ability of *Cephalosporium* sp. to solubilize phosphate but also indicated that all the four fungi tested had the same property. Noteworthy was the fact that *Penicillium* sp. released the maximum amount of phosphorus, amounting to nearly four times that of individual bacteria. Since the number and density of nodules are greatly stimulated by phosphorus[8], the results of the present study indicate the role of nodular surface fungi as probable agents in the mobilization of phosphates in situ from the soil into the nodules[9].

Zusammenfassung. Es wird nachgewiesen, dass an den Wurzelknöllchen von Leguminosen Schimmelpilze vorkommen, die Calciumphosphat abbauen und offenbar die Bildung der Wurzelknötchen selber fördern.

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Studies on the Kinetic of Alkaline Phosphatase Reaction in Ehrlich Ascites Tumour Cells with the Use of Interference Microscopy

The use of interference microscopy in enzyme histochemistry was presented by Davies et al.[1], Barter et al.[2], and Casselman[3], who estimated quantitatively alkaline phosphatase activity in kidney and intestine brush border. The method is based on the measurement of the increase of the optical path difference (OPD) during incubation of the tissue in Gomori's medium for alkaline phosphatase. The enzymatic activity can be expressed as an increase of OPD per unit of time, which reflects the precipitation rate of calcium phosphate due to enzymatic action.

In the present work this method was applied to smears of cells with a low alkaline phosphatase activity. The Ehrlich ascites tumour cells were taken from mice 5 days after inoculation. The smears were made on cover slides 5 × 4.5 cm and fixed for 4 h in cold acetone. The incubation and measurements were carried out in the multipurpose Rose's chamber[4]. The chamber with the smears on its upper cover slide was filled with an incubation medium of the following composition: sodium β-glycerophosphate $0.02M$ (0.2–$0.002M$), $CaCl_2$ $0.04M$, $MgCl_2$ $0.005M$, in $0.05M$ barbiturate buffer pH 9.2 (8.8–9.6). OPD measurements were performed with the MPI interference microscope[5,6]. Ten cells chosen at random were measured at 15 min intervals. The incubation lasted 45 min, because within this period the OPD increase was proportional to the incubation time.

Because of a lower phosphatase activity in Ehrlich ascites tumour cells than in brush border, the error in measurements is relatively higher. The limitation of the error was obtained by OPD measurement of 10 cells in one field of view and getting eventually the mean value of the OPD increase per cell per 15 min. This value was taken as representing the enzymatic activity of a chosen group of cells in a particular incubation medium. After 45 min incubation, calcium phosphate was removed from the cells with barbiturate buffer at pH 6.8, the chamber was filled with a new incubation medium and the reaction was repeated. OPD of the same cells was measured again. It was found that incubation repeated 5 times did not lower enzymatic activity. In order to establish the effect of hydrogen ion concentration on enzymatic activity, the same cells were measured in media at different pH. Incubation was repeated on the same cells 4 or 5 times and the first and the last incubations were performed at the same pH to find out whether the enzymatic activity was lowered during the procedure or not. The results are presented in Figure 1. At pH 9.2 the enzymatic activity

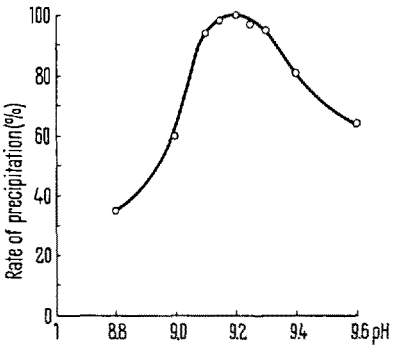

Fig. 1

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in different substrate concentrations was compared by a method similar to those described for examination of pH effect. These results are presented in Figure 2. The average value of Michaelis constant is $1 \cdot 10^{-3}$ [7]. To examine the effect of Mg^{++} ions, the OPD increase of the same cells was compared during incubation in media with and without magnesium ions. Contrary to the properties of alkaline phosphatase in kidney brush border, 15% activatory effect was noted[3].

As a preliminary experiment for further studies on the effect of different fixatives on the enzymatic activity, the influence of formaline was examined. The incubation and measurement of OPD in optimal incubation medium were performed after ordinary fixation in cold acetone, and after removal of the calcium phosphate the chamber was filled with 10% cold neutralized formaline and kept in a refrigerator at 4°C for 2 h. Then the formaline was rinsed out, the same incubation medium was introduced, and the enzymatic activity of the same cells was measured. It was found that the activity was lowered by about 55% after formaline treatment.

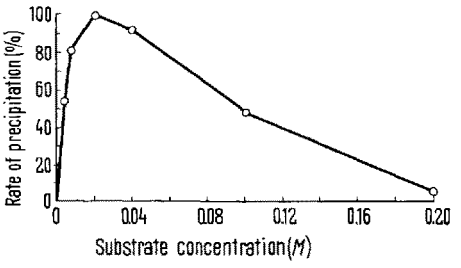

Fig. 2

Zusammenfassung. Mittels Interferenzmikroskopie wurde die Präzipitationsrate des Calciumphosphats in Ehrlich Ascites-Tumorzellen gemessen und die Beeinflussung der alkalischen Phosphataseaktivität durch diverse Faktoren untersucht.

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A Possible Way of Origin of Parthenogenetic Strains of *Dinophilus apatris* (= *D. gyrociliatus*)

Some fertilized females of *Dinophilus apatris* reproduce normally during most of their life, but at the end of their vital cycle they lay eggs which do not develop normally. The cleavage of such eggs is abnormal and they degenerate in a short time, like the eggs laid by unfertilized females. An analysis of the destiny of sperms after copulation was carried out in strains from the Leghorn Aquarium, which were bred in the laboratory under constant environmental conditions, in order to know how such degenerating eggs originate.

JÄGERSTEN[1] showed that internal fertilization occurs in *D. apatris* after introduction of the sperms through the body wall of the female and called it hypodermic fertilization. JÄGERSTEN, however, did not investigate the behaviour of the sperms after their introduction into the female's body. The histological control of many capsules containing individuals in various stages of development showed the passage of the sperms from the male to the female. Such a passage occurs immediately after hatching when the development of the female is not yet completed.

Figure 1 shows the section of a capsule where male and female embryos are copulating and the sperms are passing from the male to the female. The histological examination of two hundred females at different ages showed that sperms, after the coelomic cavity has been reached, group around the gonad. The ovary can be well identified also in very young females soon after copulation has ended (Figure 2). An evaluation of the number of male and female eggs produced by isolated and fertilized females during their whole life cycle showed that a female is able to produce, on the whole, 40–50 eggs as a minimum and 120–130 as a maximum. The number of sperms, on the contrary, ranges between 100 and 80. This is the reason why some normally prolific females lay non-developing eggs at the end of their life. Evidently these females, in spite of the fact that they have finished their sperm supply, carry on the production and laying of eggs that remain unfertilized.

Cytological control shows in fact that the number of sperms is low (Figure 3) in the coelomatic cavities of fe-

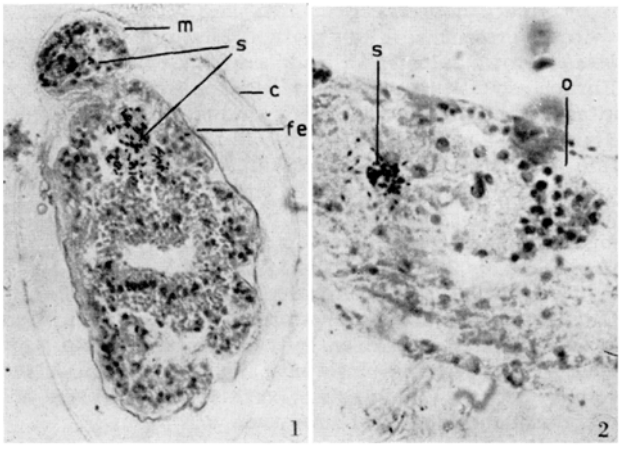

Fig. 1. Fertilization: capsule with the female embryo and the male; fertilization has almost been completed by the male.

Fig. 2. Young female: the sperms received from the male are ready for fertilization.

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