SACKLER et al.[15,16] present evidence indicative of a prenatal relationship between maternal thyroidal imbalances and subsequent childhood schizophrenics. Psychologically, THOMPSON[17] has reported that prenatal anxiety in laboratory animals increases the emotionality of progeny. KEELEY[18], similarly, noted that aberrant endocrine activity in pregnant females could possibly impair fetal response systems and influence postnatal behavior. Split-litter techniques may help to further define prenatal, maternal-fetal and postnatal, maternal-progeny relationships. In conclusion, the study demonstrated effects of maternal isolation prior to and during pregnancy on developmental growth rates and body weights of the offspring. These changes may have been in part precipitated by catabolic effects of maternal hyperadrenocorticalism on fetal metabolism. No change was noted in the fertility and fecundity of isolated mothers.

Résumé. L'isolement prolongé de souris femelles ralentit le développement et l'augmentation de poids de leurs progénitures. Aucun effet n'a été observé sur la fertilité ou la fécondité maternelle.

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Growth Response of Nodule Bacteria to Phytohemogglutanin

Since the discovery of blood agglutinising property of phytohemogglutanin (PHA) present in the seeds of *Phaseolus vulgaris*, quite an amount of information has accumulated in regard to its nature, mode of action and occurrence. Most of the experiments so far conducted with PHA have, however, been restricted to animal cells, particularly blood cells. As the chief source of this substance is legume seed[1], it would be of considerable interest to know the action of the substance on the nodule bacteria which are invariably present in intimate symbiotic association with the root system of these plants. Also, the action of PHA on the growth and reproduction of bacteria in general is yet to be studied. In this context an attempt has been made firstly to study the action of chemically pure crystalline PHA and that of crude aqueous seed extract of *P. vulgaris* on the growth behaviour of *Rhizobium japonicum* and *R. phaseoli*.

Materials and methods. The following 3 strains of *Rhizobium* belonging to 3 different cross inoculation groups were used: B.U. 8, *R. phaseoli* originally obtained from Australia and also re-isolated as pure culture from *P. vulgaris*; B.U. 1, *R. japonicum* isolated from *P. aureus*; B.U. 8/110, *R. japonicum*, received from U.S.A.D. as strain No. 110, a slow growing strain.

Aqueous, crude seed extracts were prepared at 10 °C from 20 g of seed of 2 varieties of *P. vulgaris*, the 'Red Rajmah' and the white seeded 'Solan Selection'. After centrifugation at 2000 rpm the volume of the supernatant fraction was made up to 1000 cm^3 with Y. W.-Mannitol broth. 1 ppm solution of crystalline PHA in Y. W.-Mannitol was used. Out of these, 500 cm^3 of each sample was kept for 20 min at 15 lb pressure to denature PHA. The remaining 500 cm^3 of each sample was rendered bacteria-free by passing it through a C. G. Jena filter No. 5. These were inoculated with equal volumes (2 cm^3) of bacterial suspension from 10 h old culture. The optical density of the broths were determined with a Heligere absorptiometer at 470 m/U. Direct cell count was also made with a haemocytometer under a phase contrast microscope.

Experimental results. The growth rates of any one strain of *Rhizobium*, determined from any one of these 2 procedures followed, i.e. optical density measurements and direct cell count coincided with each other. Growth curves were, therefore, prepared by plotting growth rates against time factor (Figures 1, 2 and 3). The generation time was determined from the log period of the growth curves and is presented in the Table.

The relative effects of seed extract and pure crystalline PHA on growth rates have been indicated against normal growth curve of the 3 different strains of *Rhizobium* in Figures 1, 2 and 3 respectively.

Discussion. The results obtained from the above experiments clearly indicate that crystalline PHA added to the non-nitrogenous medium decreased both the generation time and the lag phase. Consequently, both the magnitude and the duration of the log period were proportionately increased. PHA, therefore, is capable of inducing mitogenetic effect to *Rhizobium* cells, similar to its effect on the blood W.B.C. It is significant to note, however, that the relative responses to PHA of the 3 different strains of *Rhizobium*, associated with the 3 different hosts, were different. These differences again were related to the relative amount of PHA present in the seeds of the 3

Strain No.	Generation time with PHA	Normal
B.U. 1	1 h 25 min	1 h 50 min
B.U. 8	1 h 35 min	1 h 50 min
B.U. 8/110	1 h 29 min	2 h

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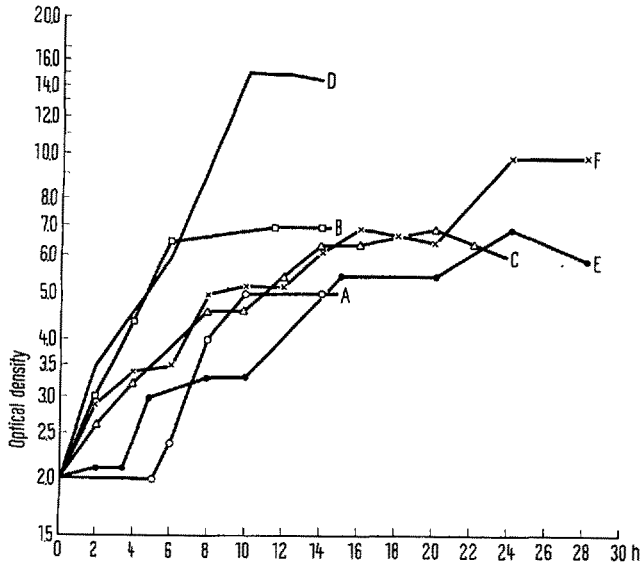

Fig. 1

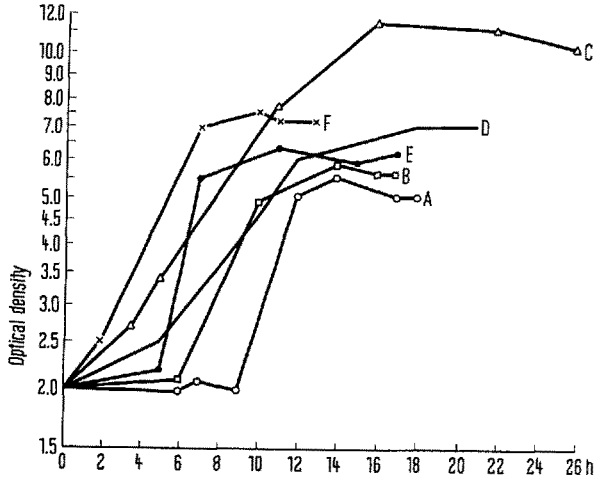

Fig. 2

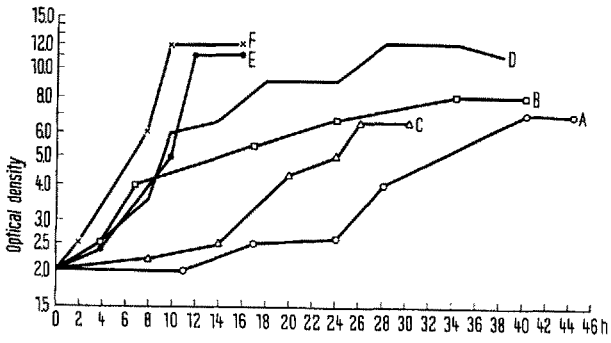

Fig. 3

Figs. 1–3. Growth curves with crystalline PHA (B); red seed extracts autoclaved (E), non-autoclaved (F); white seed extracts autoclaved (C); non-autoclaved (D); control (A). Optical density is plotted against the time of incubation. (1) Strain No. B.U. 1. (2) Strain No. B.U. 8. (3) Strain No. B.U. 8/110.

hosts. The least response to PHA was with *P. vulgaris*, *Rhizobium* where the host seed contained the highest amount of PHA. Conversely, response to PHA was maximum with the *Rhizobium* of *P. aureus*. Seeds of this latter species did not contain PHA. The soybean *Rhizobium* was intermediate in its response, as soybean seeds are reported to contain only a smaller amount of PHA[2,3]. Therefore, the differential response of the 3 Rhizobial strains to PHA depended upon the degree of tolerance achieved by the Rhizobial cells in nature to different kinds of host cells. Such parallel relationship is to be found between plant cells and c-mitosis inducing power of colchicine.

By comparing the growth curves, of aqueous seed extracts with that of crystalline PHA, it would appear that the differences are more due to the quantity of PHA present in the crude extracts than on other factors.

The response of the 3 different Rhizobial strains to autoclaved seed extracts differed conspicuously from the non-autoclaved ones. Obviously the PHA is denatured after autoclaving. The deviations observed in these curves, as against the normal growth curve, should be due to availability of extra nutrition in the autoclaved seed extract over the Y. W.-Mannitol medium. The polyauxical growth curve obtained by using crude extracts, both autoclaved and non-autoclaved, suggest stepwise utilisation of nutrients available in these extracts.

It is interesting to observe that the growth curves of *R. phaseoli* in non-autoclaved seed extracts of both the red and the white seeds are almost similar. Although these white seeds contained a higher percentage of PHA, it is probable that the amount that will be required to reach the threshold concentration to bring about the differences between the growth curves of the 2 varieties i.e., the white and the red would be less in the former than in the latter.

The divergence observed in growth curves of the 3 different strains of *Rhizobium* when grown in seed extracts, both autoclaved and non-autoclaved, as against their uniform behaviour when grown in Y. W.-Mannitol with crystalline PHA, must be due to the presence of other thermostable growth factors in the seeds.

The PHA present in *P. vulgaris* seeds does not determine the cross inoculation barrier between *R. phaseoli* and *R. japonicum* is indicated by the fact that PHA in no way inhibits the growth of *R. japonicum* in vitro. Further experiments on these lines are in progress.

Zusammenfassung. Es wird gezeigt, dass kristallines Phytohaemagglutanin (PHA) oder Rohextrakt aus PHA enthaltenden Zuckerbohnen (*Phaseolus vulgaris*) das Wachstum von *Rhizobium* dadurch beeinflusst, dass die Lagphase der Wachstumskurve verkürzt, die logarithmische Phase aber verlängert wird. Die PHA-Wirkung ist deutlicher bei den *Rhizobium*-Linien, die in den Wurzelknöllchen des Wirts an PHA nicht adaptiert sind.

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