

be signaled to the dendrites through a change of potential or another rapid event. The shortest latency measured from the application of a stimulus to the first action potential of a chemosensitive sense cell was found to be 1 and 5 msec for salt and sugar solutions¹². This almost immediate reaction would not be understandable if the molecules of the stimulus reached the dendritic membrane by diffusion.

The effects of alcohols and hydrocarbon amines on the feeding behavior of the blow fly have been found to be caused by inhibition of and injury to the unknown chemosensitive membrane rather than by stimulation of a 'rejection fiber'¹³. The viscous material at the tip of the taste hair offers a new approach in the search for the chemosensitive membrane¹⁴.

Zusammenfassung. Licht- und elektronenmikroskopische Studien der chemorezeptiven Spitze von Geschmackshaaren wurden bei den Fliegen *Phormia regina*, *Calliphora vicina* und *Stomoxys calcitrans* durchgeführt. Die Geschmackshaare dieser Fliegen ähneln sich strukturell bis auf einen ausgeprägten Öffnungs- und Schliessmechanismus, der für die Geschmackshaare von *Stomoxys calcitrans*

lichtmikroskopisch nicht nachgewiesen werden konnte. Die Resultate werden hinsichtlich der Lokalisation der chemosensiblen Membran diskutiert.

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¹⁴ We thank Dr. W. G. BODENSTEIN, Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, for his help in the photography and in the preparation of the manuscript.

Effects of Isolation on Maternal Aggressiveness and Body Growth Rates of Offspring

As a consequence of the behavioral abnormalities and endocrine imbalances caused by isolation stress, the present study was designed to determine the effects of prior periods of prolonged isolation on maternal fertility and fecundity and maternal-fetal and offspring interrelationships. Various investigators have reported that isolation stress caused behavioral abnormalities such as head-shaking¹⁻⁴, heightened locomotor activity^{4,5}, nervousness and aggressiveness^{1,3,6,7} in mice. Physiologically, evidence of hyperadrenocorticalism has been noted in isolated mice^{4,7} and rats⁸ accompanied by indications of thyroidal imbalances^{4,7,9} and reductions in gonadal weights^{4,7}. Recent reports⁹ claim that isolation caused pituitary-gonadal stimulation and that isolation-induced aggressiveness is dependent on an intact pituitary-gonadal axis with the adrenals exerting a modulating influence. Although other hormonal factors and balances, in addition to sex steroids, are involved in oogenesis as well as fertility and fecundity relationships, if pituitary-gonadal function is stimulated by isolation one might reasonably anticipate, in addition to organ and secondary sex characteristic changes, higher levels of fertility and fecundity in the isolated females.

A total of 60 albino females averaging 19 g were divided into test and control groups. All test or isolated mice were housed singly in stainless steel cages (6.5 × 10 × 7 inches) as opposed to control mice which were maintained in groups of 2/cage. The laboratory recognizes the sensitivity of animal growth and development to such environmental and physical stimuli as temperature¹⁰, noise¹¹ and handling¹². Parameters measured were changes in litter size, pup mortality and the developmental growth rates of the young. Body weights and neck-twitch responses were recorded weekly; locomotor activity¹³ and aggressiveness at various intervals during

the 6½ month isolation period. After observation of consistent significant increases in locomotor activity and aggressiveness, all test and control females were subjected to mating-behavior interaction studies with proven albino males on 4 consecutive days for 1½ h intervals. Each female was then mated individually for 6 days. All males were then removed, control females regrouped into the original pairs and then separated shortly before parturition. By this procedure, each test and control female could thus raise and nurse her own litter, and isolated females were only in contact with another animal for the relatively short mating period. All females were checked daily for date of birth and size of litters. En masse weighings and counts were made weekly at the end of the 1st, 2nd and 3rd weeks. All offspring were weighed, weaned and sexed at 4 weeks of age.

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In agreement with previous findings, isolation produced increased restlessness, hyperactivity, viciousness and the abnormal head-twitch response in the test female mice. Analyses¹⁴ of aggressive tendencies during the mating-behavior studies revealed a statistically significant increase in the number of test females attacking males at the first trial. A significant increase was also noted in the total number of attacks made by the test versus control females during the 4 day mating-behavior study. Examination of fertility and fecundity findings (Table I) indicated no significant differences between the pregnancy frequencies and the respective litter sizes of the test versus control females at parturition and weaning. The depression noted in the average litter size of the control group at weaning could be attributed to chance. One control mother cannibalized all pups during the first week, and the second control mother's progeny died as she developed a severe hip infection. Chi-square analyses of mortality in the offspring from birth to weaning revealed no statistically significant effects on offspring viability between the 2 groups. It is apparent that the data failed to demonstrate any influence of prior periods of maternal isolation on birth frequency, litter size and offspring viability during the 4 week nursing and pre-weaning period.

Table II demonstrates that the body weights of the pups obtained from the isolated mothers at the completion of the 1st and 2nd weeks were markedly lower but not statistically significant. After the 3rd week, the 15%

decrease in the individual body weights of the offspring of the isolated mothers was significantly decreased. When weaned at 4 weeks of age, significant decreases were similarly noted in the body weights of the male and female progeny obtained from the isolated mothers with the greater effects shown by the female offspring. The findings thus indicated inhibited developmental growth rates in these offspring.

It is of interest that study of aliquot populations of male and female offspring from the isolated mothers at 4½ weeks of age showed heightened evasiveness and significantly increased locomotor activity in open-field tests. Significant decreases were also noted in total leucocyte and eosinophil counts and thymus and splenic organ weights of such offspring, suggesting increased adrenocortical activity (to be published).

Questions to be resolved and defined, therefore, are the respective dominance and contributions of maternal endocrine imbalances and/or maternal abnormal behavior on prenatal embryological and postnatal developmental growth patterns of the offspring. Differences in maternal behavior, nursing care and degree of lactation may likewise produce postnatal modifications in the offspring.

¹⁴ G. W. SNEDECOR, *Statistical Methods* (Iowa State College Press, Ames 1946).

Table I. Effects of maternal isolation on pregnancy frequencies and litter sizes of isolated mice

Group	No.	Adult females		Total No. of offspring				Average litter size	
		No. of pregnant females	% pregnancy	Total No. at birth	Total No. at weaning	No. dead by weaning	% mortality	At birth	At weaning
Isolated mice S.E.	27	19	70.4	143	119	24	16.8	7.5 ± 0.4	6.3 ± 0.4
Control mice S.E.	27	20	74.1	150	115	35 ^c	23.3	7.5 ± 0.3	5.8 ^d ± 0.6
P value		> 0.90 ^a				0.15 ^a		> 0.90 ^b	

^a Chi-square. ^b Students *t*-test. ^c Corrected by removal of 2 control mothers (21 offspring dead, 15.4% mortality). ^d Corrected by removal of 2 control mothers, average litter size, 6.4 weanlings/litter.

Table II. Effects of maternal isolation on average pup and weanling weights of isolated mice

Group	At end of 1st week		At end of 2nd week		At end of 3rd week		At end of 4th week			
	Total No. young	Average body wt. (g)	Total No. young	Average body wt. (g)	Total No. young	Average body wt. (g)	Males		Females	
							No. weanlings	Average wt. (g)	No. weanlings	Average wt. (g)
Isolated mice S.E.	132	3.3 ± 0.2	124	5.2 ± 0.2	122	7.2 ± 0.4	57	9.9 ± 0.3	62	9.6 ± 0.2
Control mice S.E.	120	3.7 ± 0.1	116	5.7 ± 0.2	115	8.5 ± 0.4	67	11.0 ± 0.4	48	11.3 ± 0.4
% difference		— 10.8		— 8.8		— 15.3		— 10.0		— 15.0
<i>P</i> value ^a		0.09		0.12		< 0.01		0.02		< 0.001

^a Student's *t*-test.

SACKLER et al.^{15,16} present evidence indicative of a prenatal relationship between maternal thyroidal imbalances and subsequent childhood schizophrenics. Psychologically, THOMPSON¹⁷ has reported that prenatal anxiety in laboratory animals increases the emotionality of progeny. KEELEY¹⁸, 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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¹⁵ A. M. SACKLER, M. D. SACKLER, R. R. SACKLER and Co TUI, J. clin. exp. Psychopath. 12, 224 (1951).

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Growth Response of Nodule Bacteria to Phytohemagglutinin

Since the discovery of blood agglutinating property of phytohemagglutinin (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 seed¹, 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 cm³ with Y. W.-Mannitol broth. 1 ppm solution of crystalline PHA in Y. W.-Mannitol was used. Out of these, 500 cm³ of each sample was kept for 20 min at 15 lb pressure to denature PHA. The remaining 500 cm³ 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 cm³) of bacterial suspension from 10 h old culture. The optical density of the broths were determined with a Heligere absorptiometer at 470 m/μ. 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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