Table II. Total body length of *Polydesmus angustus* Latzel reared on different food media at a constant temperature of 23 °C with 90% + 5 RH in constant darkness

Rearing media	Mean length ± S.E. (mm)	Number measured
Oak litter	25.7 ± 3.7	43
Beech litter	26.9 ± 4.8	49
Birch litter	26.5 ± 3.5	45
Oak + birch litter	18.5 ± 4.5	25
Oak + beech litter	22.6 ± 3.2	28
Beech + birch litter	20.5 + 4.5	20

Zusammenfassung. Unter konstanten Temperatur-, Feuchtigkeits- und Lichtbedingungen entwickelt sich der Millipedier *Polydesmus angustus* rascher und wird grösser, wenn im Fallaub einzelner Baumarten gezüchtet, als in gemischtem Laub. Bei wechselnden Bedingungen (0–30 °C, wechselnder Feuchtigkeit und natürlichen Lichtverhältnissen) sind diese Unterschiede nur noch für reine Eiche statistisch gesichert.

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PRO EXPERIMENTIS

Degree of Allogeneic Histoincompatibility Assayed by the Hemolytic Plaque Technique

In 1963 BAIN et al.[1,2] reported that when human peripheral blood leukocytes were cultured together considerable numbers of blastoid cells appeared; in addition, about half of the cells incorporated thymidine into DNA. The stimulation of blastogenesis was not observed in leukocyte combinations of monozygotic twins. These responses have been considered as a primary immunological response, in vitro, and appear to be related to the genetic incompatibility between individuals. These findings were confirmed by others[3,4] and also extended to squirrel monkeys[5], rabbits[6], rats[7,8] and mice[7,9]. CHAPMAN and DUTTON[6] showed increased radioactive thymidine into DNA of lymphoid cells when spleen or lymph node cell suspensions from 2 unsensitized outbred rabbits were incubated together and indicated that the cells responsible for thymidine incorporation were large, undifferentiated cells. Further, DUTTON[7] showed that the enhanced DNA synthesis was correlated with histoincompatibility of mice strains.

It has been well established that phytohemagglutinin (PHA) transforms a population of small lymphocytes into blastoid cells[10,11]. Recently HOLM and PERLMANN[12] demonstrated that PHA-treated cells had cytotoxic potential against target cells. Hence, there exists a possibility that allogeneic lymphoid cells, cultured together, may interfere with the ability of cells to mature into antibody-producing cells. An attempt was made to elucidate the cell interaction between incompatible normal lymphoid cells by utilizing the hemolytic plaque assay.

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Plaque-forming cells in combinations of parents and F_1 hybrid

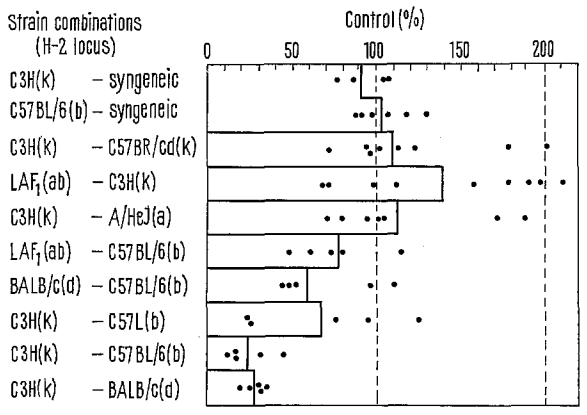
Strain combinations	H-2 loci	No. of experiments	2C/A+B (per culture dish)			Control (%)[a]	
LAF_1 – LAF_1	ab – ab	6	1420/2040 3150/3740	3490/3740 1375/1124	2230/2980 2990/2980	(Mean 95.7	± S.E.) 10.2
LAF_1 – A/HeJ	ab – aa	9	760/2110 1800/2230 3150/3675	880/1955 1040/2145 2290/2600	3220/2610 3680/3415 1790/2760	78.0	9.4
LAF_1 – C57L	ab – bb	6	2650/2735 1130/2285	3060/5320 1630/5480	4060/5630 3260/4705	62.4	9.3
A/HeJ – C57L	aa – bb	7	270/940 630/1700 220/4285	260/785 1460/2750	530/975 710/4120	32.5	7.1

[a] Percent of controls = $2C/A+B \times 100$ where C represents the number of PFC arising from a total of 1.0×10^7 strain A and B spleen cells cultured together in equivalent numbers. A represents the number of PFC arising from 1.0×10^7 strain A spleen cells cultured separately. B represents the number of PFC arising from 1.0×10^7 strain B spleen cells cultured separately.

6 inbred strains and a F_1-hybrid were used in this study, namely, C3H, C57BL/6, C57BR/cd, BALB/c, A/HeJ, C57L and LAF_1 (F_1-hybrid of A/HeJ and C57L). All mouse strains were obtained from Jackson Laboratory at 6 weeks of age and kept in our laboratory for 4 to 6 months. The in vitro system described by MISHELL and DUTTON[13] was used to assay for the maturation of mouse spleen cells into antibody-producing cells. Hemolytic plaque-forming cells (PFC), assessed by the technique of JERNE et al.[14], served as an indicator for the degree of interaction between cells. This interaction, referred to as percent of control, was determined by following ratio: $2 \times C/A+B \times 100$ where C (1.0×10^7 cells) represents lymphoid cells from strain A (0.5×10^7 cells) and B (0.5×10^7 cells) cultured together and A (1.0×10^7 cells) and B (1.0×10^7 cells) represent cells cultured separately.

Considerable variations, shown in the Figure, were seen in mixed cultures of allogeneic spleen cells. Certain combinations, such as C3H (H-2 locus – k) – C57BL/6 (b) and C3H (k) – BALB/c (d), however, showed consistent inhibition of PFC. A slight but not significant stimulation of PFC was also observed in certain strain combinations, namely C3H (k) – C57BR/cd (k), C3H (k) – A/HeJ (a) and C3H (k) – LAF_1 (ab). The degree of suppression or stimulation appeared to be associated with histoincompatibility at the H-2 locus; that is, suppression of PFC was observed between strongly incompatible lymphoid cells and no change or a slight stimulation was observed among strains with weak histoincompatibility. Variability in the percent of control was prominent in combinations with supposedly weak histoincompatibility. It was also observed that spleen cells of C3H were reduced much greater in number and responded much less to sheep erythrocytes than those of other mouse strains in our culture system (to be published). The stimulation of PFC in some mixed cultures, therefore, might have been due to other factors such as substances released from effete cells.

If suppression of PFC is the result of incompatible lymphoid cell interaction, parent-F_1 hybrid combinations should provide additional information concerning this interaction. In theory cell interaction between parental strains should be a two-way, while parental strain – F_1 hybrid combination should be a one-way interaction because hybrid spleen cells supposedly share all of the parents' antigenic determinants. Suppression of PFC in the latter, therefore, should be less than those between parental strains. Results shown in the Table indicated that maximum suppression was obtained with combinations of parental strains, whereas parent-hybrid combinations were intermediate in their suppression. The precise nature of cell interaction between allogeneic strains is not presently understood. It is unlikely that antigenic competition plays a major role since the antigenicity of sheep erythrocytes is much stronger than that of allogeneic spleen cells. Similar inhibition of PFC in mixed spleen cell suspensions from mice which were immunized with sheep erythrocytes previously and normal allogeneic mice was reported by FRIEDMAN[15].



Plaque-forming cells in mixed cultures of allogeneic spleen cells. Each point represents 1 experiment. Percent of control = $2 \times C/A+B \times 100$ where C represents the number of PFC arising from a total of 1.0×10^7 strain A and B spleen cells cultured together in equivalent numbers. A represents the number of PFC arising from 1.0×10^7 strain A spleen cells cultured separately. B represents the number of PFC arising from 1.0×10^7 strain B spleen cells cultured separately.

The present study is consistent with the results of DUTTON[7] who utilized thymidine incorporation into DNA as an indicator of cell interaction. Several explanations for the observed suppression of PFC are possible. One possibility is allogeneic inhibition[16]; MÖLLER and MÖLLER[17] suggested that the same basic process may be responsible for both inhibition and stimulation of lymphoid cells. A second possibility is that blastoid cells which appear after contact of allogeneic lymphoid cells may act cytotoxically against other lymphoid cells, including precursors of PFC. Blastogenesis in mixed cultures of allogeneic lymphocytes has been established in humans[1–4], and stimulation of DNA synthesis assessed in the interaction of lymphoid cells of incompatible mouse strains[7,9]. The relationship between stimulation of DNA synthesis and suppression of PFC is not presently known; some insight may be gained from the observations of others[12,18] that blastoid cells induced by pretreatment of normal lymphocytes with PHA impair target cells. Accordingly, allogeneic lymphoid cells in mixed cultures may transform to blastoid cells and undergo cell replication. These blastoid cells may then exhibit cytotoxic effects, in most instances, on precursor lymphoid cells, leading to suppression of PFC[19].

Zusammenfassung. Es wurden in vitro die Wechselwirkungen von Milzzellen verschiedener allogener Mausstämme untersucht. Als Indikator für die cytotoxische Wirkung der lymphoiden Zellen wurde die Fähigkeit dieser Zellen, in vitro Antikörper zu bilden, herangezogen (Methode von MISHELL und DUTTON[13]). Es hat sich dabei gezeigt, dass bei ausgeprägter gegenseitiger Histoinkompatibilität die Zahl der Plaque formenden Zellen erniedrigt ist. Bei geringer Histoinkompatibilität war die Zahl der Plaque formenden Zellen unverändert oder leicht erhöht.

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