embryonic area of *D. lacteum* raised on a cover-slip is different from that of *Planaria torva*[1], being rather similar to the division observed in natural polyembryony[2]. The divided syncytia of *D. lacteum*, like those of *P. torva*[1], do not develop into larvae. It seems that the factor which induces polyembryony (sufficiently thin layer of the cocoon content spread all over the cover-slip) makes, in turn, both separation and rounding of the buds difficult. Nevertheless, the beginning of the differentiation of the blastomeres into the embryonic pharynx could be observed in the separated areas (Figure 2). In the most flattened syncytia, neither is part of the syncytium surrounded by the cells of provisional ectoderm, nor does the division of the inner part of the syncytium take place. Nevertheless, some blastomeres differentiate into the embryonic pharynx (Figure 3) and are able to perform normal functions.

Summing up, it should be stated that the embryonic development of *D. lacteum*, starting from an egg-cell, to a form in which the differentiation of germ-layers takes place, can be investigated in vivo on cover-slip cultures. This method allows a deeper experimental analysis of the development of *Tricladida*.

Résumé. En culture entre lamelles de verre, on a examiné expérimentalement le développement embryonnaire de *Dendrocoelum lacteum* et observé le phénomène de la polyembryonie expérimentale analogue à la polyembryonie naturelle.

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Pharmacogenetic Factor in the Convulsive Responses of Mice to Flurothyl

The existence and the importance of genetically-determined variations in sensitivity to drug action is emphasized in the new subject of pharmacogenetics as defined and reviewed by KALOW[1] and MEIER[2]. Among the reports of differing drug responses between inbred strains of mice there are several involving drugs affecting the central nervous system, but few dealing with convulsants. MEIER et al.[3] determined the reactivity of 4 strains to pentylenetetrazol in an attempt to correlate this convulsive response with strain-characteristic 'arousal levels' as reflected in locomotor activity. Of these 4, the DBA/2 mice had the shortest latency and longest duration of convulsions with intraperitoneal pentylenetetrazol, while C57BL/6 mice had by far the longest latency. Similarly, we have reported that DBA/2 mice differed considerably from the C57BL/6 strain and from 2 lines of random-bred albino Swiss mice, having the lowest latency for myoclonic jerk and clonic seizure in response to the volatile convulsant ether flurothyl[4]. In the present research we sought to confirm these earlier observations and further test the correlation of convulsive and locomotor responses using certain of the same strains as in the studies cited above.

Measurements of the convulsive response to flurothyl (hexafluoordiethyl ether, Indoklon[5]) were conducted by the continuous inhalation method of TRUITT et al.[6], with the exception that a 15% rather than a 10% concentration of flurothyl in ethanol was used. Latencies (in sec) for first myoclonic jerk, for first sustained (3 sec) clonic seizure and for tonic extensor seizure were recorded while adding 0.05 ml of flurothyl every 30 sec. Measurements of locomotor activity were made upon groups of 4 mice placed without injection or other treatment into photocell-type actometers (Woodard Research Corp., Herndon, Virginia). Total counts for a 30 min period were recorded for 6 groups from each strain.

Inbred mice of the C57BL/6, DBA/2, C3H/An, CBA and BALB/c strains were obtained from Cumberland View Farms (Clinton, Tenn.). A random-bred Swiss-Webster albino strain (NLW) was obtained from the National Laboratory Animal Co. (Creve Cœur, Mo.). All mice were received at 30 days of age and were convulsed at 38–45 or 49–56 days of age. Others tested for locomotor activity were between 6 and 12 weeks old.

Responsiveness of the 6 strains to flurothyl was determined twice, using 20 different mice of each strain in each of the 2 replications. Tests were confined to a 3 h time span (08.30–11.30) to avoid circadian variation[7]. The results (Figure) show considerable differences between strains that were rather consistent between replications. There was significant heterogeneity among variances for the different strains: the C3H strain showing a particularly low variance while the BALB/c strain was at the high extreme. The data were transformed to reduce the heterogeneity in order to permit analysis of variance. For the myoclonic jerk latencies the transformation used was $\log(x - 50)$, and for the remaining data the transformation was $\log(\times - 100)$. As variances were still slightly short of the desired degree of homogeneity by the test of DAVID[8], an α-level of 0.01 rather than 0.05 has been used in the analyses of variance and intergroup comparisons in accord with the recommendation of MCNEMAR[9].

Variance analyses which were conducted across the 2 replications for each of the 3 convulsive response criteria revealed highly significant differences between treatments (strains + replications) and among strains ($p < 0.005$, Table I). There was one significant replication effect but no significant interactions (strain × replication). The Duncan multiple range test[10] was applied to the means of combined replications for all strains at each of the 3 response criteria. Significant ($p < 0.01$) differences among the 6 strains are indicated in Table II.

[1] W. KALOW, *Pharmacogenetics, Heredity and the Response to Drugs* (W. B. Saunders, Philadelphia 1962).
[2] H. MEIER, *Experimental Pharmacogenetics* (Academic Press, New York 1963).
[3] G. W. MEIER, J. L. HATFIELD, and D. P. FOSHEE, Psychopharmacologia *4*, 81 (1963).
[4] W. M. DAVIS and O. L. WEBB, Experientia *20*, 291 (1964).
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[6] E. B. TRUITT JR., E. M. EBERSBERGER, and A. S. C. LING, J. Pharmac. exp. Ther. *129*, 445 (1960).
[7] W. M. DAVIS and O. L. WEBB, Medna exp. *9*, 263 (1963).
[8] H. A. DAVID, Biometrika *39*, 422 (1952).
[9] Q. MCNEMAR, Psychol. Bull. *54*, 361 (1957).
[10] W. F. FEDERER, *Experimental Design* (MacMillan, New York 1955), p. 26.

These findings show (as did our earlier report[4]) that the DBA/2 mice had a shorter latency to myoclonic jerk and clonic seizure, and presumably responded at lower brain levels of the drug, than did any other strain tested. This deviation occurred at an age (7–8 weeks) when the familiar audiogenic seizure susceptibility of this strain has almost completely disappeared. Although the DBA strain had a significantly lower tonic seizure latency than 3 of the strains, their responses by this criterion were not distinguishable from 2 others. The highest degree of variation among strains occurred in the case of the tonic seizure response. While emphasizing the high convulsive reactivity of the DBA/2 strain, we must also call attention at the other extreme to the consistently low reactivity of the BALB/c and C57BL/6 strains. This is in accord with the report of MEIER et al.[3] regarding the C57BL/6 strain, but inconsistent relative to the pentylenetetrazol response of the BALB/c strain.

By comparison of Tables II and III it may readily be seen (and confirmed by rank-correlation statistics) that there is no consistency between convulsive and locomotor responses of the several strains. It must be concluded from our data, as well as from that of MEIER et al.[3], that interstrain variation patterns for 'behavioral arousal' measured by locomotor reactivity are not correlated with patterns of 'brain excitability' measured by convulsive reactivity. The agreement of these data in the main with previous experiments[3,4] supports the conclusion that reproducible strain-characteristic differences in reactivity to convulsant drugs do exist between various strains of laboratory mice[11].

Table I. Analysis of variance of flurothyl convulsive latencies in 6 strains of mice

Response	Source of variation	D.F.	Sum of squares	Mean square	F
Myoclonic jerk	Among treatments	(11)	1.048	0.095	6.14[a]
	Among strains	5	0.778	0.156	10.04[a]
	Between replications	1	0.043	0.043	2.77
	Interaction	5	0.227	0.045	2.92
	Error	228	3.540	0.016	–
	Total	229	4.588	–	–
Clonic convulsion	Among treatments	(11)	2.942	0.267	4.85[a]
	Among strains	5	1.552	0.310	5.63[a]
	Between replications	1	0.719	0.719	13.07[a]
	Interaction	5	0.671	0.134	2.43
	Error	228	12.563	0.055	–
	Total	229	15.505	–	–
Tonic convulsion	Among treatments	(11)	0.844	0.077	9.96[a]
	Among strains	5	0.793	0.159	28.78[a]
	Between replications	1	0.002	0.002	> 1
	Interaction	5	0.049	0.010	1.27
	Error	228	1.748	0.008	–
	Total	229	2.592	–	–

[a] Significant at $p < 0.01$; all others non-significant ($p > 0.01$).

Table II. Interstrain comparisons of convulsive latencies by the Duncan test on combined replications

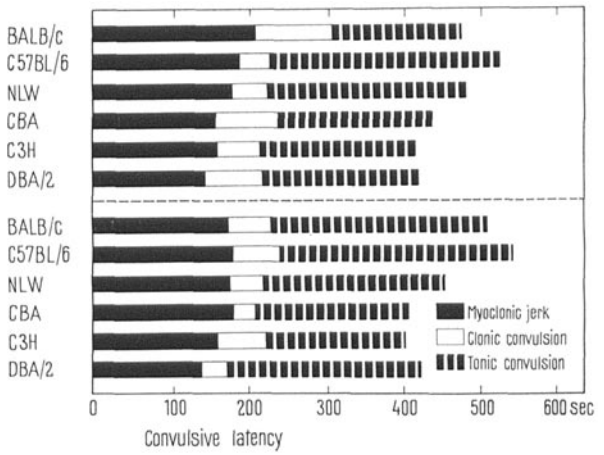
Response	Means of log-transformed data[a]					
	DBA/2	C3H	CBA	NLW	BALB/c	C57BL/6
Myoclonic jerk	1.960	2.051	2.071	2.105	2.122	2.126
Clonic seizure	1.901	2.041	2.038	2.030	2.163	2.106
Tonic seizure	2.500	2.492	2.506	2.568	2.584	2.640

[a] Values grouped by underline do not differ significantly from each other ($p > 0.01$); differences between values not sharing underline ar significant ($p < 0.01$).

Table III. Interstrain comparison of locomotor activity by the Duncan test

Mean counts per 30 min[a]					
CBA	BALB/c	DBA/2	C3H	C57BL/6	NLW
1841	1963	2098	2139	2944	3442

[a] Values underlined together do not differ significantly; those not sharing underline differ at $p < 0.05$.



Comparison of convulsive response latencies to flurothyl of 6 mouse strains in 2 replications.

Résumé. La sensibilité convulsive a été determinée dans 6 groupes de souris par les réactions de l'ether hexafluorodiéthyle, convulsivant chimique. La lignée DBA/2 fut très sensible, tandis que les lignées C57BL/6 et BALB/c se montrèrent les moins sensibles. La sensibilité convulsive ne présenta pas de corrélation positive avec l'activité locomotrice.

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