

caused a slight increase in the growth rate compared with saline injection controls; this growth rate increase, however, was less than equimolar aspartic acid injections. A startling exception occurred when N-2(6-methylheptyl) aspartic acid, at a dose of 500 mg/kg caused the death of all of the animals within 6 days. The 2 corresponding β -methyl esters showed fair increases in growth rate. In all cases, an autopsy of the animals showed no visually apparent abnormalities.

One can thus surmise from all of these results that: (1) The 5 N-alkylpyridyl aspartic acids have no observable biological effects in the systems examined. (2) N-furfuryl aspartic acid and N-2(6-methylheptyl) aspartic acid support the growth of *L. mesenteroides* P-60 in a medium deficient in aspartic acid and asparagine. The corresponding β -methyl esters not only do not support growth in absence of asparagine and aspartic acid, but show slight toxicity to the microbe in presence of asparagine and aspartic acid. (3) N-furfuryl aspartic acid, N-2(6-methylheptyl) aspartic acid, and their respective β -methyl

esters all inhibit the growth of *E. coli* 9723. This inhibition could not be reversed by 18 of the natural amino acids. (4) In general, N-furfuryl aspartic acid and N-2(6-methylheptyl) aspartic acid cause a slightly increased growth rate when injected into albino mice, however, the latter compound is lethal at a dose of 500 mg/kg. The corresponding β -methyl esters increase the growth rate measurably.

Résumé. L'acide N-furfuryl aspartique et l'acide N-(méthyl-6-heptyl)-2 aspartique permettent la croissance de *L. mesenteroides* P-60 en absence de l'acide aspartique et de l'asparagine. Ces 2 composés et leurs esters β -méthyliques s'avèrent toxiques pour *E. coli* 9723.

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Strain Differences in the Lethal Factor Exerted by Submandibular Glands Transplanted from Male Mice

Recently we postulated the presence of the lethal factor in the submandibular glands of male BALB/c mice when they were autologously or isologously grafted^{1,2}. Several factors, other than the lethal factor, have been detected from extracts of submandibular glands only of male mice³⁻⁸. Sexual dimorphism of submandibular glands of mice was first reported by LACASSAGNE⁹. The hormonal influence in sexual dimorphism of this gland has also been reported¹⁰⁻¹⁴. The nerve growth factor¹⁵ and the lethal factor¹⁶ were found to be testosterone dependent. Activities of amylase¹⁷⁻¹⁹ and renin^{20,21} in the mouse submandibular glands were found to be influenced by genetic factors as well as testosterone. As the lethal factor was demonstrated only in BALB/c mice and its nature is not yet known, an attempt was made to explore it further by comparing the lethal effects among 4 different inbred strains of mice. The preliminary report on this study was previously presented²².

Materials and methods. Adult male and female mice of the CBA, BALB/c, C3H and C57BL inbred strains were used. The former 2 strains were originally obtained from Dr. W. U. GARDNER's colony at Yale University, and the latter 2 from the Jackson Laboratory. Thereafter, all strains have been raised by sister-to-brother mating,

maintained under uniformly controlled environment and provided with Purina Lab Chow and water ad libitum^{2,23}. The female rats used were the Wistar strain obtained locally. They weighed approximately 200 g when used.

The submandibular glands were removed from the donor mice and immediately transplanted i.p. into the host animals. Autografting was performed immediately following bilateral submandibular-sialoadenectomy. The donor and host relationships were autologous, isologous, allogeneic, and heterogeneic. The mortality rates of the host animals were observed. All the dead animals and also the surviving animals which were sacrificed 30 days following transplantation were subjected to autopsy.

Results. The mortality rates of the host mice receiving i.p. either a single, one-half, or one-quarter submandibular gland isograft, when compared among the 4 inbred strains of mice, demonstrated a clear-cut strain difference, BALB/c being the highest and C3H the lowest (Table I). The female hosts exhibited higher rates of mortality than the males. However, when the female mice were used as donors of the submandibular gland isografts, no hosts died regardless of the strains used.

The mortality rates of the host mice receiving i.p. autografts of either double or single submandibular glands

Table I. Strain differences in mortality rates of host mice receiving i.p. isografts of submandibular glands

Strain of mice	Mortality rate With single submandibular gland grafts Donor to host				With $\frac{1}{2}$ gland grafts Donor to host		With $\frac{1}{4}$ gland grafts Donor to host	
	δ to δ	δ to ϕ	ϕ to δ	ϕ to ϕ	δ to δ	δ to ϕ	δ to δ	δ to ϕ
BALB/c	80/92 ^a	10/10 ^a	0/9	0/20	5/20 ^A	16/20 ^E	0/21	2/20 ^J
C57BL	2/20 ^b	17/20 ^f	0/10	0/10	0/10 ^B	4/10 ^F	0/10	0/10
CBA	3/30 ^c	11/20 ^g	0/10	0/10	0/18 ^c	5/11 ^G	0/19	0/12
C3H	0/20 ^d	9/20 ^h	0/10	0/10	0/20 ^D	2/12 ^H	0/14	0/11

Statistical differences ($P < 0.05$): b < a, c < a, d < a, g < e, h < e, g < f, h < f, b < f, c < g, d < h, B < A, C < A, D < A, H < E, A < E, C < G, J < E.

were compared (Table II). No females died. In male groups, strain difference was not exhibited with double gland autografting, but was evident with single gland autografting: the CBA and C3H strains showed no mortality at all.

The mortality rates of the host mice receiving i.p. allogeneic grafts of single submandibular glands were compared (Table III). When BALB/c males were used as donors, the mortality rates of the hosts were highest regardless of the strains of mice used. The C57BL strain of mice tended to show higher rates of mortality when used as hosts.

The mortality rates of female rats receiving i.p. heterologous grafts of quadruple submandibular glands from male or female BALB/c donor mice were 10/10 and 0/11, respectively.

Most of the host mice and all of the host rats were dead within 24 h following transplantation. When the animals survived for longer than 4 days, they lived in good health until the experiments were terminated. The autopsy findings in both the host mice and rats were comparable and were also the same as previously reported^{2,24}.

Discussion. The present experiments confirm the presence of the lethal factor in the submandibular gland grafts obtained from adult male mice. The submandibular

glands transplanted from male mice of the BALB/c, C57BL, CBA and C3H strains, exhibited lethal effects on host animals, but strain differences were also demonstrated. The lethal effect was exerted in the autologous, isologous, allogeneic, and heterogeneic transplantation systems.

The intensity of the lethal factor was strongest in the submandibular glands of the BALB/c strain and lowest in the C3H strain. The susceptibility of the different strains of mice to the lethal factor seemed to vary, the C57BL mice being most susceptible. There were no significant differences between the CBA and C3H strains of mice. This is probably due to their close genetic relationships²⁵.

Rats receiving male BALB/c grafts were found to be highly susceptible to the lethal factor exerted by heterogeneic submandibular grafts. They died within 24 h following transplantation and showed strong local and systemic hemorrhagic symptoms identical to those previously observed in the mouse^{2,24}. It is well known that either allogeneic or heterogeneic graft tissues will be rejected by the host animals, but it has not been reported that transplantation of a normal organ to normal host animals results in killing the recipient animals. Therefore, the effect exerted by the submandibular grafts in the present experiment was due to some substance released from the grafts to the hosts that was lethal. This lethal factor previously found to be testosterone dependent¹⁶, was now found to be genetically influenced²⁶.

Table II. Strain differences in mortality rates of host mice receiving i.p. autografts of submandibular glands after bilateral submandibular-sialoadenectomy

Strain of mice	Sex	Mortality rate	
		With double grafts	With single grafts
BALB/c	male	10/10	10/10
	female	0/10	—
C57BL	male	12/12	10/12
CBA	male	10/10	0/9
C3H	male	9/11	0/20
	female	—	0/10

Zusammenfassung. Der Lethalfaktor von Submandibulardrüsen-Transplantaten wurde bei Mäusen verschiedener Mutanten, C3H, CBA, BALB/c und C57BL, beobachtet. Die Intensität des Lethalfaktors war am stärksten in Transplantaten von BALB/c Mäusen und am geringsten in denen von C3H Mäusen. Die C57BL-Variante war am anfälligsten für den Lethalfaktor.

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Table III. Mortality rates of allogeneic host mice receiving single submandibular gland grafts

Donor	Host	Mortality
♂ BALB/c	♂ BALB/c*	80/92
	♂ C57BL	10/10
	♂ CBA	9/10
	♂ C3H	8/10
♂ CBA	♂ CBA*	3/30 ^a
	♂ BALB/c	1/10
	♂ C57BL	8/22 ^b
♂ C57BL	♂ C57BL*	2/20
	♂ BALB/c	1/10
	♂ CBA	1/11
♂ C3H	♂ C3H*	0/20
	♂ BALB/c	2/10

* Data in isograft system. Statistical difference: $a < b$ ($P < 0.025$ without Yates' correction).

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