the upper limit being about 2096 cm^{-1}, are indicative of an increased population of *gauche* rotamers, that is, a transition to the liquid crystalline phase (*12*). However, the relation between frequency and conformational disorder is not linear (*13*). This necessitates the use of a simple two-component overlapping band model, analogous to that employed in ^2H NMR studies (*4*, *6*), to obtain the proportions of gel and liquid crystal phases (*14*).

Figure 2 shows temperature plotted against frequency of the CD_2 symmetric stretching band in the spectra of the live cells and the isolated membranes. Below 20°C, membranes of live cells are in the gel phase; between 20° and 34°C they undergo a transition to the liquid crystalline phase. On cooling the system exhibits slight hysteresis but reverts to the gel phase at low temperature. At the growth temperature (30°C) the frequency is 2093.3 cm^{-1}. Use of the two-component model indicates that at least 50 percent of the lipids are in the liquid crystalline phase at this temperature.

The isolated membranes also undergo a gel to liquid crystal phase transition. However, while the widths of the transitions are the same for isolated membranes and live bacteria, the transition of the former occurs at a temperature about 4°C higher. At the growth temperature the frequency of the CD_2 stretching band in the spectrum of the isolated membranes is 2092 cm^{-1}. That is, the liquid crystalline phase content is only about 20 percent, as compared to the 50 percent content of the live cell membranes. However, the general form and width of the transition and the liquid crystalline content of the isolated membranes are in excellent agreement with the results of a recent ^2H NMR study of *A. laidlawii B* grown at 30°C on perdeuteromyristic acid (*4*).

A comparison was also made between transitions of live cell and isolated membranes from a culture grown at 25°C. The reduction in growth temperature results in decreased fluidity in both systems, to the extent that the isolated membranes were effectively gel phase (frequency, 2090 cm^{-1}) at 25°C. However, the band was at 2092 cm^{-1} in the spectrum of the live cells, indicating a liquid crystalline phase content of at least 20 percent and confirming the trend observed with the culture grown at 30°C.

These data confirm that, at the growth temperature, membranes isolated from *A. laidlawii B* grown on saturated fatty acids in the presence of avidin are highly ordered, and that only a small proportion of their lipids is in the liquid crystalline phase. However, in the membranes of live *A. laidlawii B* a much higher proportion of the lipids is in the liquid crystalline phase at the growth temperature. Hence, under these growth conditions, data on conformational order obtained from isolated membranes are not always directly applicable to the live microorganism.

Since both series of spectra were recorded with the same buffer, there appear to be two potential causes for the difference between live cells and isolated membranes: (i) the process of extraction changes the intrinsic properties of the membranes and (ii) part of the mechanism for regulating membrane fluidity is extrinsic to the membranes, and hence is eliminated by membrane isolation.

DAVID G. CAMERON
ADÈLE MARTIN
HENRY H. MANTSCH
Division of Chemistry,
National Research Council of Canada,
Ottawa, Ontario, Canada K1A 0R6

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Trypsin Inhibition by Mouse Serum: Sexual Dimorphism Controlled by Testosterone

Abstract. *The trypsin inhibiting activity in the serum of male mice is substantially greater than that in females. In five strains of mice and two large groups of interstrain hybrids this difference ranged from 14 (in ICR mice) to 55 percent (in DBA mice). Castration of males significantly decreased the serum trypsin inhibiting activity, whereas the administration of testosterone restored the activity to its original level. Administration of testosterone to female mice increased the activity to a level similar to that in males of the same strain. Because almost all the change in inhibiting activity occurred in the electrophoretic α-1 region, α-1-antitrypsin is probably responsible for this effect.*

Several protease inhibitors occur in mammalian serums (*1*). One inhibitor in human serum, known as α-1-antitrypsin (or α-1-protease inhibitor), is responsible for approximately 90 percent of the trypsin inhibitory activity of the serum (*2*).

A homologous protein has been identified in mouse serum (*3*). Differences in the trypsin inhibiting activity of serums from inbred strains of mice have been observed (*4*), although the magnitude of these differences has probably been overestimated (*5*). Higher trypsin inhibiting activity in males than in females has been reported (*4*), but detailed measurements on large groups of animals have not been made. In this report, we demonstrate that the trypsin inhibiting activity in the serum of male mice is substantially higher than that in the serum of females. We also show that androgens are a major determinant for the higher trypsin inhibiting activity in males.

To measure the trypsin inhibition of many individual samples we developed a semiautomatic quantitative method (*6*) that allows measurements of 50 to 100 samples per day. All strains of mice (*7*) showed a significant male-female difference in trypsin inhibiting activity, rang-

ing from 14 percent in ICR mice to 55 percent in DBA mice. All animals, with males and females in separate cages, were kept under identical conditions. After they were received, they were held for 1 week before blood samples were taken in order to avoid variability due to shipping conditions. Vesell (*8*) has shown that seemingly trivial environmental factors can lead to substantial elevations of inducible enzymes in mice.

We also tested some serum samples from crosses between strains and from animals at different ages. The age of animals at the time of testing, in the age range that we used, did not influence the trypsin inhibiting activity: serum from mice that were 6 to 8 weeks old had the same inhibiting activity as serum from 8-month-old retired breeders of the same strain.

Sufficient individual samples were available to allow a statistical comparison of only three strains and two hybrid groups. We used the two-sided Student's *t*-test for paired or unpaired samples for all subsequent comparisons. Levels for males and females were calculated separately. In the males, the order of inhibiting activities from lowest to highest was (DBA × C57BL)F_1, C57BL, ICR, DBA, and (SEC × CBA)F_1; the difference between C57BL and ICR was not statistically significant ($P = .11$). In the females the order of increasing inhibiting activities was (DBA × C57BL)F_1, C57BL,

Table 1. Serum trypsin inhibiting activity in several strains of mice and two large groups of hybrids. The values are expressed as milligrams of trypsin inhibited by 1 ml of serum (± standard deviation).

Strain	*N*	Males	*N*	Females	Male-female difference (%)	Significance of the male-female difference* *P*
C57BL/6J	28	1.62 ± 0.13	20	1.17 ± 0.10	38	<.001
ICR	38	1.68 ± 0.17	39	1.48 ± 0.22	14	<.05
DBA/2J	14	2.14 ± 0.09	15	1.38 ± 0.145	55	<.0001
P/J	2	2.52 ± 0.10	3	1.68 ± 0.17	50	<.001
129/J	3	1.65 ± 0.09	2	1.37 ± 0.39	20	<.02
BALB/c	3	1.75 ± 0.19	3	1.18 ± 0.22	48	<.05
(DBA × C57BL/6J)F_1	102	1.44 ± 0.25	99	0.96 ± 0.28	50	<.0001
(SEC/1Re × CBA, SEC/Rl × CBA)F_1	246	2.22 ± 0.123	258	1.66 ± 0.167	34	<.0001

*Two-sided *t*-test.

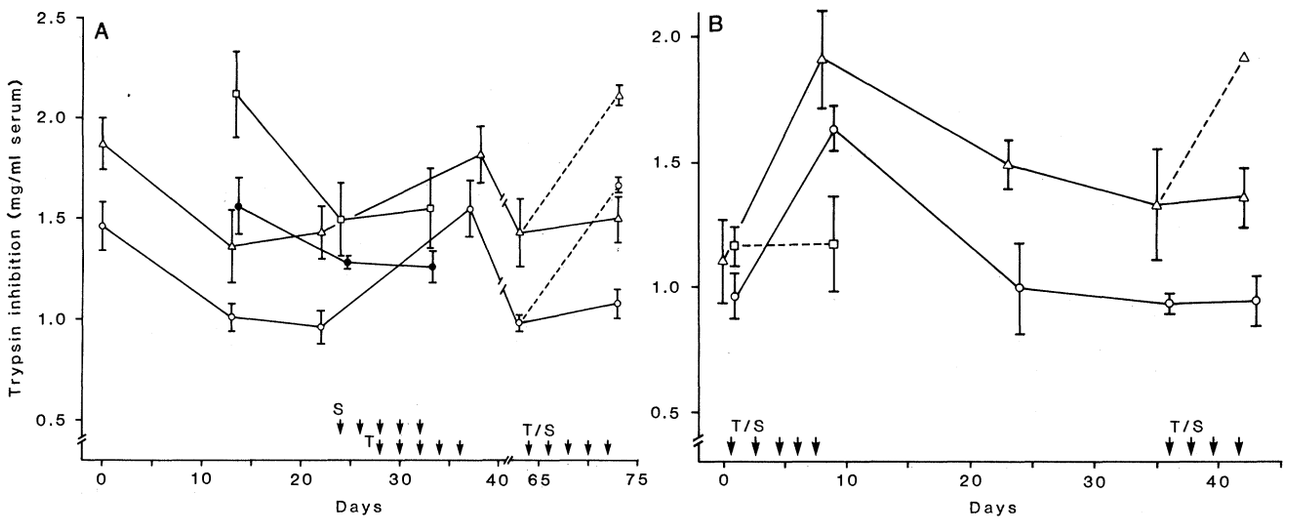

Fig. 1. (A) Serum trypsin inhibiting activity in ICR and C57BL/6J male mice. The activity was measured initially on day 0, and then the animals were castrated on day 1 under sodium pentobarbital and ether anesthesia. Trypsin inhibition was measured on days 13 and 22, then, starting on day 28, testosterone propionate (0.25 mg in 0.25 ml of sesame oil) was injected every other day (↓ T) for 9 days (until day 37). Inhibition was measured on day 38. After another 26 days (day 64) the same groups were injected with 0.25 ml of sesame oil for 9 days every other day (↓ S). Symbols: △, ICR mice, castrated ($N = 6$); ○, C57BL/6J mice, castrated ($N = 11$). (Two animals of each group were injected with 0.25 mg of testosterone for the same 9 days.) □, ICR controls ($N = 4$); ●, C57BL/6J controls ($N = 3$). These control mice were also castrated, but they received 0.25 ml of sesame oil instead of testosterone. Castration decreased the trypsin inhibiting activity in all four groups: 1.46 ± .12 to 1.01 ± .08 (C57BL/6J); 1.87 ± 0.13 to 1.36 ± 0.22 (ICR); 2.16 ± 0.19 to 1.49 ± 0.18 (ICR control); 1.56 ± .14 to 1.28 ± 0.03 (C57BL/6J controls). All values are in milligrams of trypsin inhibited by 1 ml of serum. Testosterone administration increased the inhibiting activity significantly ($P < .001$) [by 0.39 ± 0.18 mg/ml (ICR) and 0.59 ± 0.16 mg/ml (C57BL), respectively] to precastration levels [from 1.43 ± 0.14 mg/ml 1.82 ± 0.14 mg/ml (ICR) and from 0.96 ± 0.08 mg/ml to 1.55 ± 0.14 mg/ml (C57BL)]. Four weeks later (day 64) all animals had trypsin inhibition activities close to the postcastration levels before testosterone had been given. Both groups were then used as their own controls: they received 0.25 ml of sesame oil by subcutaneous injection every other day for 9 days, except for two animals in each group that received 0.25 mg of testosterone. Again, sesame oil had no effect on the activity whereas testosterone injections (---) lead to a significant ($P < .001$) increase [by 0.67 ± 0.09 mg/ml (ICR) and 0.61 ± 0.16 mg/ml (C57BL)] in inhibition values from 1.43 ± 0.17 mg/ml to 2.12 ± 0.05 mg/ml in ICR and from 0.98 ± 0.04 to 1.67 ± 0.04 in C57BL/6J. Three male mice (two C57BL/6J and one ICR) that received sham operations showed no significant difference in inhibiting activity 13 and 27 days following the procedure (data not shown). (B) Serum trypsin inhibiting activity of female mice in response to testosterone. Two groups of mice (six C57BL/6J, ○; four DBA/2J △) were given 0.25 mg of testosterone propionate in sesame oil subcutaneously every other day for 8 days (↓ T). A control group of six C57BL/6J (□) mice received 0.25 ml of sesame oil (↓ S). Blood samples were taken before the injections were started and on day 9 the mice were left without treatment and further blood samples were taken on days 24 and 35. Then, both groups received 0.25 ml of sesame oil except one animal in the DBA group that received 0.25 mg of testosterone. Only this animal showed an increase in trypsin inhibition value (---). The testosterone-treated group showed a significant ($P < .002$) increase in serum trypsin inhibiting activity from 1.1 ± .21 to 1.91 ± 0.19 mg/ml (DBA) and 0.96 ± 0.15 to 1.63 ± 0.09 mg/ml (C57BL). Sesame oil injections had no effect on the trypsin inhibiting activity.

DBA, ICR, and (SEC × CBA)F_1. However, the difference between DBA and ICR females was not statistically significant (P = .076), whereas all other differences were significant with P values of .001 or smaller.

Because of the striking difference betwen males and females, we studied the influence of androgenic hormones on the trypsin inhibiting activity. In one experiment we examined the effect of castration and subsequent testosterone administration on the serum trypsin inhibiting activity in male mice and in another we measured the effect of testosterone on the inhibiting activity in the serum of females.

Trypsin inhibiting activity decreased significantly in male mice after castration (Fig. 1A). The inhibiting activity remained low for at least 9 days. However, when the same animals received testosterone (*9*), their inhibition values returned within 1 week to the precastration level.

Two groups of female mice of inbred strains were given a subcutaneous injection of 0.25 mg of testosterone propionate in 0.25 ml of sesame oil every other day for 8 days. Blood samples were taken 1 day before the first testosterone injection and on day 9 (Fig. 1B). The inhibiting activity increased significantly in all females to approximately the level of the males of the same strain. When the testosterone administration was discontinued the trypsin inhibition returned to the starting values. A second series of testosterone injections in the same animals reproduced the increase in the trypsin inhibiting activity, whereas the controls treated with sesame oil showed no significant elevation (Fig. 1B).

We also examined the electrophoretic zone with which the increase in trypsin inhibiting activity was associated. We found that the changes that occurred after testosterone administration in females or after castration in males were almost entirely due to inhibitors in the α-1 zone (Fig. 2). This suggests that most of the increase was probably due to α-1-antitrypsin.

In humans, α-1-antitrypsin is an acute phase reactant that increases in response to several stimuli. Diethylstilbestrol, danazol, a synthetic androgen, and estrogenic steroids (*10*) are among the compounds that trigger an increase in α-1-antitrypsin in humans. There is no difference in the serum trypsin inhibiting activity or α-1-antitrypsin concentration between healthy men and women (*11*).

The mouse is well known for its sexual dimorphism with respect to several proteins including some that are secreted by the liver (*12*). Such quantitative sexual dimorphism occurs in the major urinary proteins. Male mice excrete larger quantities of these proteins in their urine than females, and females can be induced to secrete more of the proteins by the administration of androgens (*13*). Castration of male rats leads to a decrease of α-2-urinary-globulin concentration (*14*).

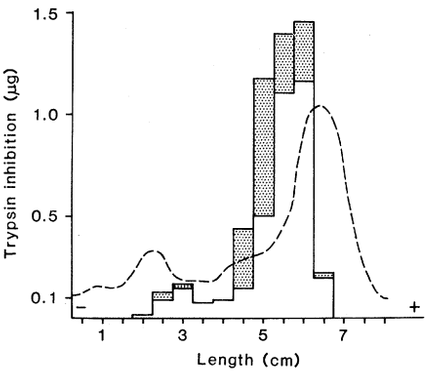

Fig. 2. The trypsin inhibiting activity of the electrophoretic α-1 and α-2 zones of serums of a C57BL/6J female before (unshaded areas) and after testosterone treatment (shaded areas). The anode (+) was at the right. The origin was at 0. The interrupted line indicates the protein profile. Ninety-eight percent of the increase in trypsin inhibiting activity was in the α-1 zone. In castrated males the decrease in trypsin inhibiting activity was also predominantly in the α-1 zone (*15, 16*).

Our studies add another example to this group of androgen-controlled proteins in the mouse. Protease inhibitors including α-1-antitrypsin, are well suited for studies of the induction of such proteins because they can be conveniently measured in serum and can be assayed sequentially in the same animal more easily than tissue enzymes.

As practical consequence of our results, we suggest that the sexual dimorphism as well as the difference in serum trypsin inhibiting activity between strains should be taken into account in experimental designs in which protease inhibition may be an important variable.

FRIEDRICH KUEPPERS
JULIE MILLS

Department of Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

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6. A Stasar III (Gilford) equipped with an enzyme programmer and enzyme calculator was programmed to dispense 0.2 ml of a trypsin solution (0.1 mg/ml) and 0.5 ml of benzoyl-arginine-*p*-nitroanilide (BAPNA, Sigma) (1 mg/ml water) into reaction vessels containing 0.5 ml of a 1.0 percent solution of mouse serum in 0.1*M* tris containing 0.007*M* $CaCl_2$-$2H_2O$, *p*H 7.6. This assay mixture was taken up automatically into the cuvette of the photometer and the hydrolysis of BAPNA at 30°C was followed at 410 nm. The trypsin inhibiting activity was calculated by comparing the remaining activity with a standard curve prepared with known concentrations of trypsin. The trypsin inhibiting activity was expressed as milligrams of trypsin inhibited by 1 ml of serum. The trypsin was active-site–titrated by the method of T. Chase and E. Shaw [*Biochem. Biophys. Res. Commun.* **29**, 508 (1967)] and was found to be 66 percent active. Bovine trypsin (lyophilized) was from Boehringer, Mannheim.
7. Mice of different strains were obtained from the following sources: C57BL/6J, 8 weeks old, were from S. Mann, Temple University. The ICR mice, 6 to 8 weeks old, were from S. Mann and from Perfection Breeders, Inc., Douglasville, Pa. The following were retired breeders (6 to 8 months old) from the Jackson Laboratory: DBA/2J, P/J, 129, BALB/c, and C57BL/6J. Serum samples from (DBA/2 × C57BL/6)F_1 were from S. Lewis, Research Triangle Park, N.C. Serum samples of (SEC/1Re × CBA)F_1 and (SEC/Rl × CBA)F_1 hybrids were from R. Popp, Oak Ridge National Laboratory. (SEC/1Re × CBA)F_1 and (SEC/Rl × CBA)F_1 were initially treated as two groups; however, when their trypsin inhibiting activity was found to be identical, they were combined.
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15. In mouse serum, trypsin inhibiting activity is present in the electrophoretic α-1 and α-2 region (approximately 85 to 15 percent). To locate the regions with the increased inhibiting activity, we analyzed paired serum samples from the same animal before and after castration or before and after testosterone treatment by electrophoresis and measured the trypsin inhibiting activity of the electrophoretic zones. Both serums (40 μl of each) of a pair of samples were subjected to electrophoresis on agarose plates (10.1 by 8.2 cm; thickness of the agarose layer, 0.18 cm, sodium barbital buffer 0.1*M*, 0.005*M* $CaCl_2$, *p*H 8.65) for 2 hours at 200 V and 25 mA. After the run, 0.5-cm sections of agarose were cut and placed in test tubes and 0.25 ml of tris buffer (0.1*M* tris hydroxymethyl aminomethane, 0.005*M* $CaCl_2$, *p*H 7.6) was added to each tube. The tubes were left at 4°C for 14 hours. Trypsin inhibiting activity of the individual fraction was measured essentially as described in (*16*), except that 0.025 ml of the supernatant was added to 0.006 mg of trypsin in 1 ml of tris buffer. All other reagents were adjusted proportionally. Since the trypsin inhibiting activity had been determined in the serum sample before electrophoresis, we could compare this value with the sum of those in the individual electrophoretic fractions. We found 90 percent of the expected inhibiting activity after electrophoresis.
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