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## The Effects of Strain Differences on the Binding of Concavalian A to Mouse Sperm

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THE EFFECTS OF STRAIN DIFFERENCES ON THE  
BINDING OF CONCAVALIAN A TO MOUSE SPERM

by

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## INTRODUCTION

In the current study, lectins were used as genetic probes to determine the phenotype of the sperm from different strains of mice. Lectins are proteins of non-immune origin, which bind to specific carbohydrates (McCoy, 1986). Being that virtually all cell membranes contain carbohydrate componenets, lectins have been used to identify all types of cells.

The specificity of carbohydrate binding by lectins was first discovered by their ability to distinguish between red blood cells based on blood group specificity. In the laboratory, lectins are now used for detection of cell surface carbohydrates and cell separation (McCoy, 1986). Markers, such as flourochromes, colloid gold, enzymes, radioisotopes and biotin, have been conjugated to lectins and used as probes (McCoy, 1986).

In this study, a biotinylated lectin, Concavalian A (Con A) derived from Canavalia ensiformis, was used and binding was detected using an avidin-biotin peroxidase (ABP) complex technique (Fuccillo, 1985). The biotin labelling the lectin reacts with the avidin in the ABP complex, forming an essentially irreversible bond. The high affinity of the biotin-avidin bond allows detection of the conjugate at very low levels. Biotin, also known as Vitamin H, can be conjugated to many proteins without altering biological activity of the protein. Avidin can be conjugated to a detection marker or the detection label could be biotinylated and avidin used to bridge the two molecules together. A modification of the bridging method,

called the Avidin-Biotin Complex (ABC), was used to detect the binding of biotinylated Con A. For this technique, avidin is incubated with biotinylated enzyme at a precise ratio (Fuccillo, 1985). A three dimensional complex is formed with many enzyme molecules in each ABC. Thus, the sensitivity of the assay is amplified. This method also allows scoring with a light microscope instead of the fluorescent microscope needed for other types of lectin conjugates.

The object of the current research was to 1) determine if mouse strain differences have an effect on lectin binding 2) to investigate the pattern of inheritance of lectin binding and 3) develop a staining method detectable by the image analyzer (discussed later). It was hoped that strain differences in lectin binding could be identified and that its mode of inheritance discovered.

#### METHODS AND MATERIALS

##### Diluent

Phosphate buffer saline (PBS), pH 7.5. Consisted of 0.20 grams of KCL, 0.20 grams of KH<sub>2</sub>PO<sub>4</sub>, 2.16 grams Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O and 8.00 grams of NaCl in distilled H<sub>2</sub>O to equal 1.0 liter total volume.

##### Lectin

Concavalian A (Con A), Jack Bean agglutinin from Canavalia ensiformis, which binds to alpha-mannose and alpha-glucose (Millette, 1977). was purchased from Sigma (St. Louis, MO). To prepare for use, 1.0 ml of PBS was added to 2 mg of biotinylated Con A, resulting in a stock concentration of 2000 mg/ml. 10 ml of PBS was then added to 0.1 ml of stock biotinylated Con A producing a dilution of 20 mg/ml.

The dilute solution was then stored frozen in aliquots of 1.5 ml.

#### Avidin Biotin Complex

"ABC" concentrate (Hsu, 1981) prepared using 2.5 ml of 1 mg/ml biotin peroxidase and 7 ul of 1.5 mg/ml avidin then frozen. For staining, the concentrate was thawed and 1.0 ml PBS added.

#### AEC Reagent

AEC stock solution was prepared by dissolving 12 mg of 3-amino-9-ethylcarbazole in 1.5 ml of dimethylformamide and refrigerated until needed. AEC reagent was prepared by adding 0.5 ml of 0.1% peroxide (made by mixing 1 ml 30% peroxide in 30 ml of H<sub>2</sub>O), 4.5 ml of PBS and 50 ul of AEC stock solution. AEC produces a red precipitate as a result of the peroxidase reaction (Butterworth et al, 1985).

#### Mice

Six strains of mice were tested for the ability of their sperm to bind Con A. ICR mice were derived from breeding stock purchased from Harlan Sprague Dawley, Inc., (Indianapolis, IN). DBA, T/t, C57 and B6D2F1 (C57 x DBA) mice were derived from breeding stock purchased from the Jackson Laboratory, (Bar Harbor, ME). +/T (short tail) and +/t (normal tail) were obtained by crossing T/t males with +/+ ICR females.

#### Preparation of Sperm

The six strains of mice, two mice from each strain, were sacrificed by cervical dislocation during the same period. The sperm were stripped from the vas deferens into 1.0 ml PBS. The sperm were aspirated 5-10 times with a Pasteur pipet for mixing. 50 ul of sperm

mixed with PBS was placed at the end of a slide then positioned vertically for drying. The slides were stained or stored frozen.

#### Staining Method

For staining, 50 ul of biotinylated Con A, 20 mg/ml, was spread on the slides. The slides were incubated at 37°C in a humidified box for 30 minutes then washed with 10-15 ml of PBS. Slides were next covered with 4-5 drops of "ABC" reagent. The slides were incubated at 37°C in a humidified box for 30 minutes then washed with 10-15 ml of PBS. The slides were then covered with 4-5 drops of AEC reagent, incubated at 37°C in a humidified box and washed with 15-20 ml of PBS. After drying slides were mounted with a 50:50 mixture of glycerol and PBS.

#### Scoring Slides

Slides were scored under a light microscope equipped with a video camera. The camera data was fed into a IBM-PC equipped with an image analysis board. The sperm image appeared on the computer monitor and the cursor was moved to the regions of acrosome or midpiece. The computer assigned the intensity of the area under the cursor values from 0-63. Fifteen sperm and non-sperm areas were scored from each slide and averaged. The mean acrosome and midpiece values were subtracted from the mean non-sperm values for each mouse. This was done to account for the difference in the degree of binding to sperm regions and the non-sperm areas. The resulting values were termed net acrosome (NAC) and net midpiece (NMD). The NAC and NMD values were then subjected to one way analysis using the SPSS statistical package available on the university computer. Low numerical values indicated sparse binding and high numerical values indicated greater binding activity.

## RESULTS

The mean scores for acrosome binding is listed Table 1 and mean scores for midpiece binding in Table 2. The Con A binding activity for the acrosome region was significantly different for +/t, +/T and C57. These differences are indicated Figure 1 by an asterix. Only +/T exhibited significant difference in binding to the midpiece area (fig. 1). The C57 strain acrosome binding was not significantly different from the C57 midpiece binding. Nor did any of the midpiece values within the other strains differ from acrosome values.

In preliminary studies, five ICR slides were stained with biotinylated Con A and detected with FITC labeled avidin. The slides were then scored with a fluorescent microscope. The acrosome fluoresced brightly while fluorescence in the midpiece was faint. This procedure was done to approximate the area of Con A binding to sperm. Results with FITC labeled Con A also indicated strong acrosome binding and little binding to the midpiece. These preliminary studies contradict the results using biotinylated Con A and ABC as listed in Tables 1 and 2. Preliminary studies done with the various components in ABC indicated a nonspecific reaction between peroxidase and the midpiece region.

When comparing F1 progeny to the parental strains, it is seen in Figure 1 and Tables 1 and 2, B6D2F1 binds Con A to the acrosome region in an almost identical amount to that of the parental strains DBA and C57. The B6D2F1 strain also exhibited equal binding to the midpiece region as that of the DBA strain, but the F1 strain did have greater binding of Con A to the midpiece than C57.

It is only possible to compare +/T and +/t to the parental

strain ICR for lack of testing the parental strain T/t<sup>6</sup>. F1 progeny +/T showed decreased binding of Con A to the midpiece and acrosome regions as compared to the binding activity of the ICR strain. On the other hand, +/t had slightly greater binding activity than the parental ICR strain in the acrosome and midpiece regions.

#### DISCUSSION

The detection of biotinylated lectin with ABC was successfully analyzed with the image analyzer. The image analyzer is a useful tool in the quantification of lectin binding. It provides elimination of human error and a numerical intensity system which exceeds a system based on various degrees of "bright" staining of fluorescence utilized in past studies.

We have more confidence in the acrosome binding values than those of the midpiece. This is due to the fact that the results from the current study, utilizing the ABC detection method, contradicted the results of previous studies using FITC labeled lectin or FITC labeled avidin. It is believed the peroxidase in ABC is reacting nonspecifically with the midpiece region. As a result, other methods of staining using ABC have been investigated. New evidence shows that using Strep-avidin in ABC reduces reaction in the midpiece region (L.C. Ginsberg, Personal Communication).

The binding activity of +/T (low binding), +/t (high binding), and the remaining strains (medium binding) suggests that mouse genotypes might contain multiple alleles for acrosome binding of lectins. Medium binding might be an indication of a wildtype genotype while the high and low binding might be allelic expressions.



Further testing with a larger sample size would be helpful to confirm the dominant expression of lectin binding suggested by the F1 results of B6D2F1. In addition, +/T and +/t, F1 progeny of the ICR x T/t<sup>6</sup> cross should be compared to both parental strains to determine the pattern of inheritance of lectin binding in that cross. The results of both crosses could possibly then reveal the pattern of inheritance of lectin binding for all mice.

## References

1. Butterworth, H.B., Khong, T.Y., Loke, Y.W., and Robertson, W.B., 1985. Human Cytotrophoblast Populations Studied by Monoclonal Anti-bodies Using Single and Double Biotin-Abidin-Peroxidase, Journal of Histochemistry and Cytochemistry, 33:977-983.
2. Fuccillo, D.A., 1985. Application of the Avidin-Biotin Technique in Microbiology, Bio Techniques, 3(6):494-501.
3. Hsu, Rain,L., Fanger H., 1981. Use of the Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques, Journal of Histochemistry and Cytochemistry, 29:577-580.
4. McCoy, Jr, J.P., 1986. Contemporary Laboratory Application of Lectins, Bio Techniques, 4(3):252-262.
5. Millette, C.F., 1977. Distribution and Mobility of Lectin Binding Sites on Mammalian Spermatozoa, in The Immunobiology of Gametes, Cambridge University Press, London, 1977.

Table 1  
Net Acrosome (NAC)\* Binding

Strain	Mean	Standard Error
B6D2F1	19.0	0.0000
+/t**	25.0	2.0000
+/T**	11.0	1.0000
ICR	20.0	0.0000
C57**	12.5	4.5000
DBA	19.5	4.5000

\*NAC = NS(Nonsperm) - AC(Acrosome)

\*\* Significantly different at p=0.05 level

Table 2  
Net Midpiece (NMD)\* Binding

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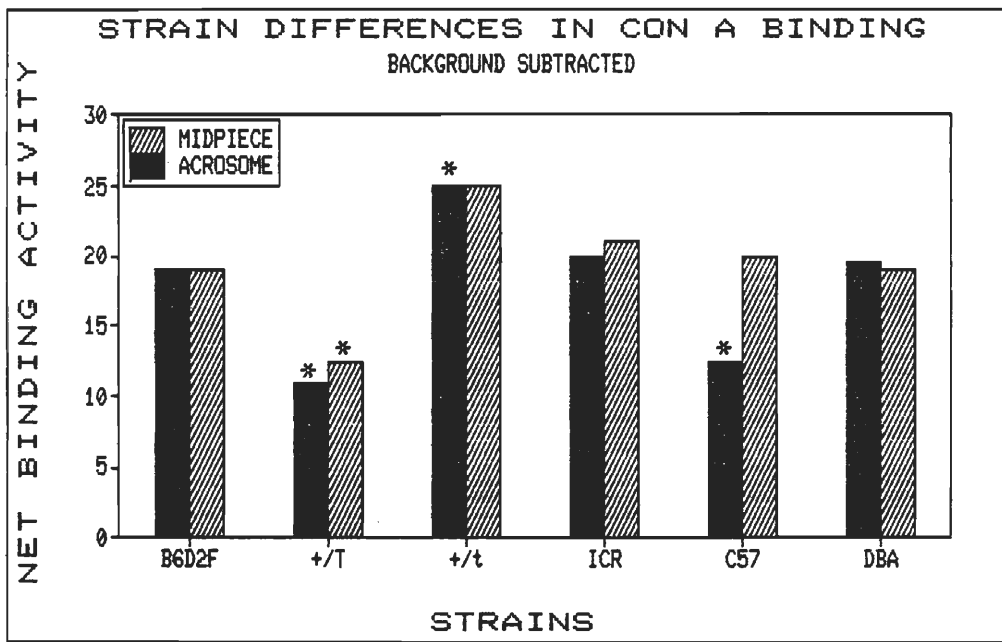
Strain	Mean	Standard Error
B6D2F1	19.0	3.0000
+/t	25.0	0.0000
+/T**	12.5	0.5000
ICR	21.0	1.0000
C57	20.0	7.0000
DBA	19.0	3.0000

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\*NMD = NS(Nonsperm) - MD(Midpiece)

\*\* Significantly different at p=0.5 level

Figure 1



\* These regions are significantly different  $p=0.05$  level as compared to the other strains.

