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ANTIGENIC CHARACTERISTICS OF SPERMATOZOA FROM BULLS, RAMS AND BOARS

I. ERYTHROCYTIC ANTIGENS IN BULL SPERMATOZOA

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Summary. We were unable to demonstrate the presence of the erythrocytic antigens of cattle either in the seminal or the epididymal spermatozoa of bulls of our Red Spotted breed. The negative results were not altered by mechanical fragmentation or by enzymic disruption of the spermatozoa.

INTRODUCTION

Soon after the discovery of the antigenic nature of erythrocytes and other body cells, attention was paid to spermatozoa. Landsteiner & Levine (1926) proved that human spermatozoa absorb antibodies from anti-A and anti-B antisera used for the determination of blood groups in man, and concluded that spermatozoa contain substances identical with or similar to the blood factors of erythrocytes. The same conclusions were drawn by several further authors, e.g. by Gullbring (1957) and Májský & Hraba (1960). In 1952, Docton, Ferguson, Lazear & Ely succeeded in absorbing on bull spermatozoa some of the antibodies used for characterizing blood groups in cattle. In this work we have tried to repeat these investigations and to demonstrate erythrocytic antigens in the spermatozoa of our brindled cattle.

MATERIALS AND METHODS

In the initial experiments during this study of the erythrocytic antigens of bull spermatozoa, we used the absorption technique of Docton *et al.* (1952). Seminal spermatozoa of eight bulls of the Red Spotted breed were tested for the absorption of the following antibodies: anti-A, B, K, I, P, Q, T, Y_1, Y_2, B′, I′, J′, K′, O′, Y′, C_1, C_2, W, F, V, J, L, M, S_1, U_2, Z, $CH_{3,4,7,9}$.

After getting negative results with the described technique, we made some changes. First, we used differently modified spermatozoa for the absorption treatment of the individual antisera, the antibodies of which were to be absorbed by the spermatozoa. The alteration was performed by repeated

freezing and thawing of spermatozoa which had been washed three times, and by enzyme treatment of their surfaces with trypsin, by the method of Morton & Pickles (1947). The incubation time of the thrice-washed spermatozoa in a 1 % trypsin solution was longer, about 4 to 20 hr. The effect of trypsin was studied microscopically. In most cases the heads of the spermatozoa were changed only slightly, but the tails were always separated from the heads.

An homogenate of bull spermatozoa was also tested for absorption of antibodies. Before homogenization, the sperm cells were washed three times, and diluted with four volumes of 0·9 % sodium chloride solution. The homogenization was performed in glass homogenizers (the spermatozoa being ground between two rough glass surfaces) and the grade of alteration was controlled microscopically. The homogenization continued until all the spermatozoa were fragmented. After homogenization, the sperm fraction was separated, diluted at a rate of 1 : 1 with 0·9 % sodium chloride solution, and, in a dilution of 1 : 2 to 1 : 256, used for the absorption treatment of the individual antisera. The supernatant was also used in absorption tests.

As the results with these procedures were negative, we used spermatozoa collected from the head and tail of the epididymis of three bulls at slaughter. Spermatozoa were obtained from the head of the epididymis by cutting it into small pieces which were afterwards extracted with the physiological solution of sodium chloride for 12 to 24 hr at 4° C. When the spermatozoa were centrifuged in the solution, the sediment contained spermatozoa which were washed three times with the sodium chloride solution and used for the absorption treatment of the antisera by the techniques of Docton *et al.* (1952). Spermatozoa were obtained from the tail of the epididymis by washing it with the sodium chloride solution.

RESULTS AND DISCUSSION

Absorption treatment of the individual antisera used for the determination of blood factors with washed spermatozoa, by the method of Docton *et al.* (1952), did not in our experiments give the results obtained by these authors. We could not determine erythrocytic antigens of cattle either in the seminal spermatozoa or in the spermatozoa from the head or from the tail of the epididymis of bulls of the Red Spotted breed. Even the use of enzymically and physically altered spermatozoa was without success. Our negative results, of which a preliminary report was given at the International Blood Group Congress in Edinburgh (Matousek, 1960), have been confirmed by Schmid (1960). For the present it is not possible to suggest a reason for these different results.

Helpern & Wiener (1961) affirm that the factors A, B and H are also present in the seminal plasma of man. We can therefore also consider the possibility that some factors are adsorbed on spermatozoa from seminal plasma. From our preliminary results it seems that only the antigenic factor J is present in the seminal plasma of the bull, but in no case was factor J shown to be present on spermatozoa. We have been unable to demonstrate the presence in seminal plasma of any of the other antigenic factors mentioned in the Methods section.

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