

Determining the Presence of Antibodies Against Sheep Red Blood Cells in a Person's Blood

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Abstract For this experiment, researchers used red blood cells from sheep as an antigen. These cells depend on the thymus gland for their development. To prepare the antigen, researchers first centrifuged the cells using a medium called medium 199. They then did another round of centrifugation at a speed of 1,000 revolutions per minute (RPM) for 600 seconds. After that, they injected the cells into animals in doses of 10 million or 2 times 10 millions cells. Researchers gave the animals a small amount of a solution called isotonic saline, approximately 0.5 millilitres.

Keywords Sheep, Antigen mongrel white rats, Blood, Lymph nodes, Endocrine system, Epiphysis

1. Introduction

To conduct the study, we used adult outbred male white rats weighing 150-180 grams and aged 2-3 months at the start of the experiment. Additionally, we also used similarly aged, adult white mice without a specific breed weighing between 18 and 22 grams at the beginning of the study. Furthermore, we used chickens at their juvenile stage of development aged between 7 and 10 days after hatching weighing between 60 and 90 grams at time of testing. To ensure the reliability and validity of results we carefully selected optimal number of animals per group using 8-10 individuals per group.

The food for the control and test groups was selected in accordance with guidelines for maintaining laboratory animals. Additionally, the research conducted on these animals followed the rules of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Strasbourg, 1986).

To conduct the study, guinea pigs were chosen. Their initial weights ranged between 300 and 400 grams. Initially, a procedure was performed to extract a substance from the animals. The animals were decapitated and their plasma, free of fibrinogen, was collected. Then, the plasma was placed in a special chamber maintained at -20 degrees Celsius.

While the plasma was in the chamber, it was mixed with an isotonic solution at a ratio of 1 part substance to 20 parts plasma. A drug derived from young chicken blood was used as an antigen for the chickens. For the antigenic material, they used sheep's red blood cells, specifically their erythrocytes. These cells need the thymus gland to produce

antigens. Before using them, the researchers centrifuged the red blood cells for 600 seconds at 1,000 RPM using medium 199 to prepare them for immunization.

The experimental subjects received a single injection of an antigen at a concentration of either 10^8 or 2×10^8 . The injection was given intraperitoneally, meaning it was injected into the abdominal cavity, in a volume of 0.5 ml isotonic solution.

2. Material and Methods

To determine the presence and quantitative composition of antibodies to sheep erythrocytes, 50 μ l of a normal isotonic solution was added to 96-well plates [1]. The next step was to add 50 μ l of plasma without fibrin from the experimental object to one of the wells, the mixing procedure was performed, and then titration by sequential transfer to the remaining wells. This had to be done to the point where it was necessary to leave only the last 2 rows of holes. One of the wells in its composition contained only a normal isotonic solution for mammalian blood as a control. Next, 50 ml of 1% solution of red blood cells of ungulates was poured into the already available solutions. The laboratory utensils were placed inside the thermostat for 60 minutes at a temperature of 37°C. Next, the antibody titer was calculated in the reaction of gluing and precipitation of erythrocytes. As an antibody titer, the final mixing of the drug was conditionally counted, denoted by an umbrella-shaped reaction, that is, interaction with the red blood cells of ungulate mammals. The results were demonstrated in the form of logarithms with base 2 (\log_2).

In order to provoke the phenomenon of antigenic competition, representatives of the genus *mus* were initially injected with horse red blood cells (EL) in a ratio of 1'09 as

the dominant antigen, and after 4 days, ram red blood cells were introduced in volumes 2'08 (immunizing antigen). After 4 days, the BOOK was directly connected in the plenum [3,4].

For the study, adult dogs of mixed breeds weighing between 18 and 20 grams were chosen. The dogs were frozen and placed on a specialized device on their backs for six hours. After that, the dogs were euthanized by decapitation after receiving light anesthesia with ether. Before placing them on the device, the animals were given something to drink or eat through a tube 60 minutes beforehand.

The next day, the animals received an antigen injection. Four days later, the same procedure was repeated with a second group of animals to determine the impact of stress on physiological parameters. The same methods were used to collect and analyze the data. Additionally, we monitored the level of immune system activity and any possible changes or specific characteristics resulting from exposure to different systems in the animals.

To simulate physical exhaustion in animals, they were put through forced swimming. While they were doing that, their immune systems were tested in relation to the substance being studied. Bemetil, which is a common drug for boosting physical performance, was used as a reference medication to see how well the test substance worked. It was given to the animals like the test substances, at a dose of 50 mg per kg. The animals were swimming in water that was kept between 27 and 28 degrees Celsius. To limit how long they could swim and to better identify when they were exhausted, we put mice (male, weighing 18-20 grams) under 5% weight on their tails. When the mice were totally exhausted, they couldn't swim back to the top without help for more than 5 seconds after reaching the bottom. We did experiments on the ability of serum to regenerate using the same exhaustion model from swimming. The only difference was that we gave the test substances to the mice right after they were totally exhausted. Then, we split the animals into groups and put them through a lot of exercise until they were exhausted, after an hour break. We wanted to see how the serum affected the animals' immune system after intense exercise (specifically, four days of swimming without a break, for four hours a day). We measured the number of cells that produce antibodies in the spleen on the fifth day to see the effect. We gave the animals an antigen-EB on the first day. On the first day, we also gave them test substances and a reference drug (T-activon and Immunal), and we did that every day for the next three days. We used Bemetyl as a control because it's known to help with endurance.

Radiation sickness

For this study, we used male white mongrel mice weighing between 18 and 22 grams. We performed a single total irradiation of the animals using a gamma-ray medical irradiator called a Theratron. The dose was 5 Gy, with a power of 1.2 Gy per minute (irradiation time was 20 minutes). The distance between the irradiation tube and the mouse's body was 65 centimeters, and the radiation source

was 60 centimeters away.

In this experiment, we administered the tested substances orally at a dose of 5 milligrams per kilogram for five days before irradiation and then for one month. We then evaluated the modifying effect of the substances on post-irradiation changes in several parameters, including the content of antioxidant capacity (AOC) in the spleen, antibody titer in the blood, total cell count in central and peripheral immune organs, and blood levels of erythrocytes and leukocytes. In this study, the substances were administered to the mice 5 days after they had been irradiated intraperitoneally at the above doses, and at the same time, they were immunized with EB. Five days later, they were brought into the experimental setting.

3. Results and Discussion

To induce the phenomenon of antigenic competition, we injected members of the MUS species with red blood cells from a horse as a dominant antigen in a 1:109 ratio. Four days later, we gave them red blood cells from a ram, in a volume of 2:108, as the immunizing antigen. Four days after that, we directly measured the AOC (antibody-opsonized complement) in their pleural cavities [2].

In a series of experiments, researchers used red blood cells from ungulates like sheep (EB) as a sort of antigen. These EB are dependent on an immune system part called the thymus and are used to create antibodies.

Before the experiments, the researchers centrifuged the EB cells to separate them into different parts. They used a medium called 199, and they spun the cells at 1000 times per minute for 600 seconds in a special machine.

The researchers injected the cells into the animals, or experimental subjects, in small doses. They injected either 10^8 cells (one billion) or $2 \cdot 10^8$ (two billion) cells, depending on the experiment. The animals got the cells through a small cut in their abdomen, and the researchers gave them a special isotonic solution with the cells in it.

4. Conclusions

The digital data from the dissertation were processed on a personal computer using statistical software. Using student's t-test and standard Excel software, we calculated the mean (M), standard deviation (SD), standard error (SE) and Student's T value. We also calculated p-values to determine if there was a significant difference between groups. Changes occurring with 95% confidence ($p < 0.05$) were considered significant. For accurate calculations, we took into account the assumptions specified in modern textbooks on clinical and laboratory research.

The data collected for this project were analyzed using a personal computer and statistical techniques. We employed the Student's t-test and standard Excel software to compute the mean, standard deviation, margin of error, and p-value. Furthermore, we calculated the likelihood of error and used a

confidence level of 95% to determine statistical significance. In order to ensure the accuracy of our findings, we adhered to the guidelines outlined in contemporary textbooks on the statistical analysis of clinical data [5].

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