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Blood Groups in the Coypu**I. Attempt to Obtain Heteroimmune Sera****Grupy krwi u nutrii****I. Próba uzyskania heteroodpornościowych surowic**

[With 1 Table]

I. INTRODUCTION

Recent papers by Owen (1962); Bogden & Aptekman (1962); Palm (1962); and Frenzl, Křen & Stark (1960), giving a.o. a survey of the results of many years' research on blood groups in rats, prove the manifold utility of research in this field. Those investigations served to some extent as a model for author's studies on differentiation of the antigenic blood properties of the coypu (*Myocastor coypus* Molina, 1792), undertaken at the initiative of Professor Dr. W. Herman¹⁾. Their aim is to determine the individual blood group traits of the coypu, and subsequently use them as an auxiliary element in various investigations on this animal. The present contribution gives the results of the preliminary attempts to obtain rabbit-anti-coypu red cell sera which after suitable treatment could serve as test sera for differentiation of blood groups in particular individuals.

II. MATERIAL AND METHOD

As test animals coypus and rabbits from the Brwinów experimental farm of the Dept. of General Animal Breeding, Warsaw Agricultural University were used.

Different procedures were used in taking blood: 1. incision of the foot vein after Ugorski (1962), 2. incision of the tail, 3. cardiac puncture when a larger blood quantity was required. In taking blood for erythrocytes, an aqueous solution of sodium citrate and sodium chloride was used as anticoagulant.

To obtain heteroimmune sera with antibodies for coypu red cells, every of 6 rabbits was given 10 times an intravenous (marginal ear vein) or in 7 cases an intraperitoneal injection of about 0.5 ml. packed coypu red cells. Before injection, the red cells were washed 3—4 times with physiological saline (0.92‰). The rabbits were

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divided in two groups of three rabbits each. One group (nr. 8H5, 420, 4513) was immunized with 50% suspension of erythrocytes (volumetric: 1 part erythrocytes + 1 part physiological saline), the second group (nr. 7H3, CS-37, 4517) with hemolyzed erythrocytes (volumetric: 1 part erythrocytes + 1 part aqua pro injectione). Immunization of the rabbits was performed within one month (May—June 1962). Between the first and second injection an unexpected interruption occurred, so that the first injection gave initial sensitization, while the following nine injections, given at 2-days intervals, formed immunization proper. After completion of the full immunization series, every rabbit was bled twice: on the first and tenth day from the last injection with coypu erythrocytes. The obtained sera were inactivated for 30 minutes at 56°C and subsequently cooled, frozen and preserved at temperatures from -18° to -22°C.

The agglutination tests were carried out by the test tube method, with three drops of appropriately diluted serum to one drop of a 2—2.5% suspension of erythrocyte sediment, washed 3—4 times with physiological saline (0.92%). After thorough shaking of the tube, incubation proceeded during 30 minutes at room temperature. The reactions were read macroscopically three times: after half an hour, one hour, and in the case of negative reaction again after another hour. The titres were determined by diluting the serum with physiological saline in geometric series (1:2, 1:4, 1:8, 1:16, and so forth).

III. RESULTS

In order to determine whether antibodies against coypu red cells occur in the normal sera of the tested animals, agglutination "cross" tests were made in the first stage of investigations, using red cells and sera of the particular coypus and also agglutination tests of coypu red cells with rabbit normal sera. In result of the above tests it was found that no hemagglutinins for coypu red cells were present in the mentioned sera.

After conclusion of the initial investigations, the next stage purposed to obtain heteroimmune sera by immunization of rabbits with red cells from one coypu (male, Nr. 13/61). To check the effectiveness of the performed immunization, the rabbit immune sera were subjected to an agglutination test with coypu red cells. The tests were made with the particular sera diluted 1:2, 1:4, 1:8, 1:16 and so forth with physiological saline. The general effect of production of antibodies was thus defined by determination of the titre of the particular sera in reaction with the particular tested coypu erythrocytes. The titres of immune sera in respect to the particular red cells showed in general great variance.

Table 1 gives only the limits within which the values varied. The figures in the tables denote the final reaction effect, that is the greatest dilution at which distinct, macroscopically defined, reaction with the respective red cells was still obtained.

The titres of sera from rabbits immunized with hemolyzed red cells (Nr. 7H3, CS-37, 4517) were in every case lower than in serum Nr. 8H5,

but much higher than that in sera Nr. 4513 and 420, originating from rabbits immunized with red cells in suspension.

Comparison the titres of the sera obtained on the first day (sera *I*) and on the tenth day (sera *X*) after conclusion of the immunization series showed that the differences between them varied for every rabbit. In general though one may say that a better effect (expressed in titre) was obtained in the case of the sera *X*, originating from blood taken on the tenth day after ending immunization. No comparison between serum *I* and *X* was possible in the case of the sera 7H3 and 8H5, since those *I* sera were titrated only in dilution 1:2 ... and so forth up to 1:4096.

Table 1.

Results of agglutination tests, denoting the titres of rabbit immune sera in reaction with coypu red cells.

Rabbit heteroimmune sera		Titres (from, to) of sera in reaction with coypu erythrocytes	
Number	after immunization with	<i>I</i>	<i>X</i>
7H3	hemolyzed r.c	4096 ²⁾ (—)	4096 — 16384 (11605)
CS-37		4096 (4096)	2048 — 4096 (2867)
4517		2048 (2048)	2048 — 8192 (4779)
8H5	r. c. in suspension	4096 ²⁾ (—)	4096 — 65536 (30720)
420		256 (256)	256 — 512 (384)
4513		512 — 1024 (922)	1024 — 2048 (1229)

²⁾ Sera titrated only to dilution 1:4096.

I — sera taken on the first day after the last injection.

X — sera taken on tenth day after the last injection. Average titre in parentheses.

Characteristic results were obtained in agglutination tests with red cells of particular coypu individuals, using relatively small dilutions (1:2 to 1:16) of four immune sera: 7H3—*I* and 7H3—*X*, 8H5—*I* and 8H5—*X*. The sera 7H3—*I* and 7H3—*X*, and the serum 8H5—*X* did not show agglutination reaction with some red cells at small dilution, that is high antibody concentration in those sera. These findings, however, do not give sufficient grounds for suggesting any regularity in occurrence of this phenomenon. Each of the above mentioned sera showed inhibition of the agglutination reaction in different degree (that is in different dilutions from 1:2 to 1:16), and also differently in regard to particular red cells. Especially interesting was the fact that the serum 8H5—*X* showed the prozone

while the serum 8H5—I of the same rabbit, but obtained from blood taken on the first day after conclusion of the immunization series, did not show any prozone when identical dilutions were tested against the same red cell samples.

IV. DISCUSSION

The preliminary investigations in which normal non-inactivated rabbit sera and coypu erythrocytes were used, gave very rapid and complete hemolysis of all tested red cells. These results justify the statement that in normal rabbit sera occur the species antibodies against coypus. Agglutination cross tests of coypu red cells and their sera, and tests of coypu red cells with inactivated rabbit sera were designed to ascertain if any normal agglutinins for coypu red cells were present in those sera. The tests showed that no normal agglutinins for red cells of the coypus used in the experiments were present in the tested (coypu and rabbit) sera.

To obtain rabbit-anti-coypu red cell sera containing agglutinins, two immunization methods were applied — immunization with hemolyzed coypu red cells and with coypu red cells in suspension. The immunization effects defined by agglutination titre (of the particular sera with red cells) did not show any significant differences between the two methods. On the basis of the results of the initial tests with normal non-inactivated rabbit sera and coypu red cells one could have assumed that the erythrocytes in suspension would after injection into rabbits get immediately hemolyzed *in vivo*, and that injection of hemolyzed red cells *in vitro* was thus only an artificial process, substituting *in vivo* reaction. The results of the titration of the immune sera obtained indicate that red cell antigens can with equal effect be injected in the form of suspended as well as hemolyzed red cells, the technical procedure being perhaps slightly more convenient in the second case. It is possible though that certain differences between both procedures may arise in isolation of specific fractions of the antibodies.

In connection with the results of the agglutination tests of particular heteroimmune sera with coypu red cells, some other characteristic observations deserve attention.

Every immunized rabbit showed relatively strong individual features regarding the effectiveness of antibody production. The differences between the titres of sera from particular rabbits ranged from 1:256 to 1:65536. Exceptional inability of antibody production was observed in the given case in rabbit Nr. 420, which produced antibodies of comparatively low titre (1:256 to 1:512). In contrary, the rabbits Nr. 8H5 and 7H3 showed exceptional good production of antibodies.

The titres of sera taken on the first and on the tenth day after the last injection showed differences in the reaction of particular rabbits to immunization with coypu red cells. In five cases this 10-day period contributed (to a greater or smaller extent) to an increase in immune serum titre over that of most other tested cells. In one case however (rabbit Nr. CS—37), the serum taken 10 days after the last injection showed (in respect to some of the tested red cells) lower titre than that taken on the first day after the last injection. This suggests that blood for serum production might be taken equally well at an earlier date than 10 days after the last injection.

The differences which occurred in testing the reaction of immune sera with particular red cells are presumably due to the different nature of the antibodies contained in the sera. In order to get definite information on this question we shall have to isolate the particular unispecific antibodies each reacting with only one antigenic specificity of coypu red cells, and collect a larger number of coypu blood samples.

V. SUMMARY

The general object of the investigations was to obtain iso- and heteroimmune sera which after suitable treatment could then be used as reagents to identify coypu red cell antigenic factors. The results presented in this communication refer to rabbit-anti-coypu red cell sera.

The preliminary investigations showed that in coypu and rabbit normal sera no iso- or heteroagglutinins for coypu red cells were present. Non-inactivated normal rabbit sera caused in every case quick lysis of the coypu red cells (*in vitro*).

Rabbit-anti-coypu red cell sera were obtained by means of injection of 50% suspension or of hemolyzed coypu red cells. The effects of immunization, defined by titres, did not indicate any significant difference between these methods. The titres of several heteroimmune sera, in reaction with red cells of individual coypus, showed generally great differences, ranging in extreme cases from 1:256 to 1:512 and from 1:4096 to 1:65536 respectively. Those differences show the possible presence in those sera of several, presumably specific, antibodies for coypu red cell antigenic factors.

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STRESZCZENIE

Zasadniczym celem zainicjowanych prac badawczych nad właściwościami antygenowymi krwi nutrii (*Myocastor coypus* Molina, 1792) jest wprowadzenie cech grupowych jako element pomocniczy do różnorodnych badań nad zwierzętami tego gatunku. W niniejszej publikacji przedstawiono wyniki prób uzyskania heteroodpornościowych surowic przeciw czynnikom antygenowym czerwonych krwinek nutrii. Materiał doświadczalny stanowiły nutrie i króliki, pochodzące z Eksperymentalnej Fermy S.G.G.W., Katedry Hodowli Ogólnej Zwierząt w Brwinowie.

W badaniach wstępnych przeprowadzono „krzyżowe” próby aglutynacyjne normalnych surowic nutrii z ich krwinkami oraz próby aglutynacyjne tych krwinek z normalnymi surowicami królików. Na podstawie uzyskanych wyników stwierdzono, że w badanych surowicach nie występowały normalne aglutyniny dla antygenów krwinkowych nutrii. Nieaktywowane, normalne surowice królicze w każdym przypadku wywoływały wyjątkowo szybko hemolizę krwinek nutrii.

W celu uzyskania surowic heteroodpornościowych, jednej grupie królików wstrzykiwano 50% zawiesinę krwinek nutrii, drugiej grupie krwinki zhemolizowane. Określony mianem surowic efekt uodporniania nie wykazał istotnych różnic między tymi dwoma metodami, obie więc można uważać za równie dobre. Miana poszczególnych surowic wykazały natomiast dość duże różnice, zarówno przy porównaniu ich między sobą, jak również przy porównaniu reakcji każdej surowicy z poszczególnymi próbkami krwinek nutrii. W skrajnych przypadkach, miana surowic wahały się w granicach od 1:256 do 1:512 oraz od 1:4096 do 1:65536. Różnice w wysokości miana poszczególnych surowic w stosunku do poszczególnych krwinek wskazywały na możliwość wyodrębnienia w nich poszczególnych frakcji przeciwciał, prawdopodobnie specyficznych dla identyfikowania czynników antygenowych krwi nutrii.