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RECEPTION EXPERIENCE PRECIPITATING IMMUNE WHEY FOR USE IN MEDICOLEGAL PRACTICE

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ABSTRACT

Immunization of rabbits for reception heteroimmune precipitating whey is spent. As an antigene for immunization of rabbits are used: a mix of whey of blood of the person, horned livestock, a bird, a horse and 10 % a formalin solution in the ratio 1:1, in volume of weight of the rabbit providing the high specific immune answer at 90 % of animals of 1 ml/kg. Received heteroimmune precipitating whey is strictly specific and high-sensitivity, providing their application in medicolegal practice.

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INTRODUCTION

For statement immunological reactions specific precipitating whey is necessary which should possess strict specificity and high sensitivity. The majority serological and immunological methods of researches provide application of immune whey which receive from blood of animals, immunized various antigenes, thus their activity essentially influences results of research. For reception of whey it is necessary to pick up the rational scheme of immunization of animals [1, 2, 3]. By it mean such factors, as a physical and chemical condition of an antigene, doses, ways, intervals and frequency rate of introduction of an antigene, the general duration of a cycle of immunization, application of our and immunomodulators. Search and working out effective schemes of immunization of animals, for reception of hyperimmune whey, set many scientists conducting researches in this direction [4, 5, 6]. As mark Hurn Century A.L. And Chantler S.M. (1980), results of studying of dependence anti-bodyformation from frequency rate of introduction of an antigene testify to obvious advantage Repeated immunization before the unitary scheme. Intervals between introductions an antigene choose an empirical way, and, results of experiences usually can be used only with reference to the given concrete antigene. Dependence between way of introduction of an antigene and level of antibodies in whey of blood of animals is traced.

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Principal causes of distinction of efficiency are speed from which the antigene extends from a place of an injection and probability of its passage through lymph nodes, and other bodies of immune system. According to T.Charda [1981], the same ultimate goal can be reached various ways, but anyway, the scheme of immunization offered for manufacture, should Correspond to certain requirements. The main requirement - possibility of reception of whey with high enough titer specific antibodies for rather short time interval at minimum an expenditure of antigens and other material.Reception specific immune precipitating fiber of the person of whey high titers for use in medico legal practice was the purpose of researches.

MATERIALS AND METHODS

In experiences 48 rabbits of breed of both sexes the Chinchilla and the Giant, by weight 3-3,5 kg are used. Animals contained in standard conditions in conformity with requirements of the methodical grant «Rules and methods of work with laboratory animals at experimental microbiological and immunological researches». In quality of bedding, sawdust from not coniferous breeds of trees was used. Mixed fodder and juicy forages for animals were given in cages. The data about structure and quality of a forage is stored in the laboratory documentation. The animal gave the cleared water. Animals contained in controllable conditions of environment (20-22°C and relative humidity of air of 50-70 %). In premises for the maintenance of animals 12-14 hour cycle of illumination was

supported. The temperature and humidity of air were registered daily. Any essential deviations of these parameters in acclimatization and during experiment has not occurred. Clinical survey of each animal spent daily. Carried out careful survey of the animal maintenance in a cage. Fixed the general condition of animals: feature of behavior, intensity, character of impellent activity, presence and character of spasms; coordination of movements, a tone of skeletal muscles; frequency, depth of respiratory movements; a condition hair, an integument; quantity and a consistence of faecal weights. As an antigen for immunization of rabbits birds (3rd group), horses (4th group) and 10 % a formalin solution in the ratio 1:1 which remained in the refrigerator at temperature 4-6°C in a current of 72 hours are used a mix of whey of blood of the person (1st group), horned livestock (2nd group). As control to each group immunization of rabbits on 2 pieces (only 8 pieces) It was made by whey of corresponding kinds and 0,9 % of a physiological solution (5th group). Immunization of rabbits was spent under the offered L.I.Lomovitskoy scheme (1977) to D.D.Dzhalalov and R.A.Hasanov updating (1997).

After reimmunization the positive result has been reached accordingly in 90 % and 95 %. Thus duration immunization made 3 weeks, titer received immune precipitating whey reached 1:10000, fatal cases at rabbits was not observed.At such scheme of immunization have been received rabbit hyper immune precipitating whey fiber of the person, horned livestock, a bird and a horse with high specific activity in immunological reactions. Thus immune stimulating effect at absence of toxic influence on animals, without occurrence adjuvant diseases, was reached at 90-95 % of rabbits. After the first introduction of antigenes appearance of animals from skilled groups differed nothing from appearance of animals from control group. After the second introduction small decrease in appetite and mobility of animals has been noticed. After the third introduction of antigenes on an injection place, at some animals, marked occurrence of hyperemia, palpitation increase.After reimmunization, the behaviour of skilled animals remained same languid in comparison with control animals, but serious changes in behavior and appearance was not observed.

Titer and specificity characteristics of precipitating immune sera

Groups	Titr/time precipitation		Control group		Specificity
1st: an antigene of the person (10pc)	1:100 (10)	+40 seconds	1:100	+3 minutes	Absence of deposits by one o'clock with the normal whey of blood of horned livestock dissolved in 1000 time, horses, birds.
	1:1000 (10)	+1 minute	1:1000	+6-8 minutes	
	1:5000 (9)	+3 minutes	1:5000	+10-15 minutes	
	1:10000 (7)	+6-8 minutes	1:10000	-	
2nd: an antigene of horned livestock (10)	1:100	+20 seconds	1:100	+2 minutes	Absence of deposits by one o'clock with the normal whey of blood of the person dissolved in 1000 time, horses, birds
	1:1000	+50 seconds	1:1000	+5-6 minutes	
	1:5000 (9)	+2 minutes	1:5000	+15-20 minutes	
	1:10000(8)	+4-6 minutes	1:10000	-	
3rd: an antigene of a bird (10)	1:100	+10-12 seconds	1:100	+1 minute	Absence of deposits by one o'clock with the normal whey of blood of the person dissolved in 1000 time, horned livestock, a horse
	1:1000	+40 seconds	1:1000	+4-6 minutes	
	1:5000 (10)	+1 minute	1:5000	+10-12 minutes	
	1:10000 (9)	+3-5 minutes	1:10000	-	
4th: an antigene of a horse (10)	1:100 ()	+30 seconds	1:100	+2 minutes	Absence of deposits by one o'clock with the normal whey of bloodof the person dissolved in 1000 time, horned livestock, a bird.
	1:1000	55 seconds	1:1000	+4-6 minutes	
	1:5000 (9)	+2 minutes	1:5000	+12-15 minutes	
	1:10000 (8)	+4-6 minutes	1:10000	-	

The antigene is entered into a regional vein of an ear of a rabbit three times, an interval 1 day in volume of weight of a rabbit of 1 ml/kg. Test of blood at immunized animals undertakes for 4th, 7th and 9th day after last injection. At presence precipitins in whey of rabbits titer 1:5000 and 1:10000 the blood sampling by a puncture of a cavity of heart and bleeding is made. After immunization if titer precipitating whey did not reach the worker titer it was spent reimmunization in two weeks after last immunization, unitary introduction of an antigene. Selection of blood and whey reception. The blood sampling was carried out from a regional vein of an ear of a rabbit (before procedure of a capture of blood an ear processed spirit). Blood put in the thermostat at temperature 37°C on 1 h. (For whey branch). After led selection of whey by centrifuging at 3000 rpm within 10 minutes. If necessary time of centrifuging increased. Whey stored at-20°S in a mix of boric acid of 3 % in the ratio 3 mg/1 of ml.

RESULTS AND DISCUSSION

Introduction of whey of blood of the person, and also animals and 10 % of a solution of formalin in the ratio 1:1 in volume of weight of a rabbit of 1 ml/kg led the immune to the answer only at 70 % immunizable animals after primary immunization, and at introduction of whey of blood of a bird the percent of animals with the immune answer reached 75-78.

After a blood sampling start definition of titer and specificity of the received whey. precipitating whey is considered suitable for medico legal researches if it has titer 1:10000 i.e. when at adding it to the homologous normal whey dissolved in 10000 times, the deposit drops out within 10 minutes and it does not give deposits normal isolation whey of other kinds dissolved in 1000 times within one hour.From the table it is visible that titer antibodies in the first group in 9 cases corresponded 1:5000 and in 7 cases 1:10000.

In the second group in 9 cases corresponded 1:5000 and in 8 cases 1:10000, in the third group in 10 cases corresponded 1:5000 and in 9 cases 1:10000, in the fourth group in 9 cases corresponded 1:5000 and in 8 cases 1:10000. It is established that all received precipitating whey was specific, i.e. in cultivation 1:1000 within 1st hour of reaction precipitation, except a corresponding kind, to other antigenes did not occur.

Thanks to application immunostimulating actions of 10 % of formalin are considerably reduced duration of process of immunization (22 days) and reception of highly specific whey, the exit of a target product at the expense of increase antibody production at animals with simultaneous reduction of expenditures of labour is thus raised. Also it is necessary to notice that unitary reimmunization that was possible to increase percent of positive results in 90-95 % cases.

Conclusions

Thus, by results of research effective schemes of immunization are developed for reception of the heteroimmune whey, based on an optimum combination of albuminous antigenes in a complex from 10 % by a solution of the formalin providing the high immune answer at 90-95 % of animals, considerable reduction of terms of immunization, material and expenditures of labour. Received heteroimmune whey is highly specific and is considered suitable for application in medicolegal practice.

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