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From the Department of Microbiology and Epizootology, College of Veterinary Medicine, the State Veterinary Medical Institute, Helsinki, and the Institute of Animal Breeding, Agricultural Research Centre, Tikkurila, Finland.

DETERMINATION OF THYROGLOBULIN ANTIBODIES IN OBESE STRAIN CHICKEN SERA USING CHROMIC CHLORIDE AS A COUPLING REAGENT

By

D. Sharma and *J. Tuomi*

SHARMA, D. and J. TUOMI: *Determination of thyroglobulin antibodies in Obese Strain chicken sera using chromic chloride as a coupling reagent.* Acta vet. scand. 1973, *14*, 651—656. — Chromic chloride was used for the coupling of chicken thyroid extract on human and chicken erythrocytes. With the coated cells thyroglobulin antibodies were determined in Obese Strain chicken sera. Ultracentrifugation of the sera prior to testing could be omitted. The method is more sensitive, less antigen requiring and less time-consuming than the previous tanned agglutination test.

autoimmunity; thyroiditis; chicken; passive hemagglutination; chromic chloride coupling.

Chromic chloride has been used for the coupling of thyroglobulin on red cells for determination of thyroglobulin antibodies in human population (*Aho et al.* 1971). Less thyroglobulin is needed and the test is more sensitive and less time-consuming than the previous tanned-agglutination (TA) test.

Obese Strain (OS) chicken sera are very rich in fatty substances and for the TA test to be applicable it is important to remove the fatty substances by ultracentrifuging (*Witebsky et al.* 1969). The minimum amount of serum required for conventional ultracentrifugation may not usually be available by wing vein puncture, the method generally used for collecting blood from the chicken.

This paper describes the method for coating chicken thyroid extract on red cells using chromic chloride as a coupling reagent and the determination of thyroglobulin antibodies in OS chicken sera by the method.

MATERIAL AND METHODS

Preparation of antigen

Chicken thyroid gland suspension was prepared in 0.9 % saline as described by *Janković & Mitrović* (1963). The suspension was subjected to ultracentrifugation at 69000×g for 30 min. (*Witebsky et al.* 1969) and the supernatant, the thyroid extract to be used as antigen for coating the cells, was preserved at —50°C in small vials each containing 0.3 ml. For the preparation of thyroid extract and further for preparing the dilution of thyroid extract, 0.9 % saline was used. *Jandl & Simmons* (1957) have stated that the use of normal saline gives the most appropriate results when chromic chloride is applied as a coupling reagent.

Erythrocytes

Human Rh-positive group O erythrocytes were used in the main experiment of the present study. The optimum age of human blood preserved in Alsever's solution, when used for the purpose of the test, was from 3 to 7 days. Cells from several chickens were also tried, but only those from a few chickens were found to be suitable for the test. When chicken cells could be successfully used, this appeared to be limited to the very first days after collection.

Coupling procedure

Prior to coating, the chicken thyroid extract was kept in a boiling water bath for 2 min. (*Witebsky et al.*). For coating of the cells 0.1 ml of washed, packed (human or chicken) cells, 0.5 ml of a 0.075 % solution of chromic chloride in 0.9 % saline and 0.5 ml of chicken thyroid extract diluted 1:20 in 0.9 % saline, were placed into a glass beaker at a short distance (2—4 mm) from each other. By gently shaking the glass beaker, the above-mentioned 3 reagents were allowed to mix. This mixture was well agitated for 5 min. at room temperature, then washed thrice with 30—40 volumes of 0.9 % saline, and finally the coated cells were adjusted to a concentration of 1 % with phosphate-buffered saline, pH 7.2.

Sera

Obese Strain chicken sera from 10 chickens and OS hatching eggs were received as a courtesy of Dr. R. K. Cole, Cornell University, Ithaca. From the hatching eggs, 45 chicks were reared at the State Veterinary Medical Institute, Helsinki. Sera received from Dr. Cole were used as control sera. Control serum no. 1 (control 1), was a

serum sample from which the lipids were not removed by ultracentrifuging. Control 2 was a pool of the rest of serum samples (9 samples), which was subjected to ultracentrifugation at 69000 × g for 90 min.

Blood samples were taken by wing vein puncture from OS chickens at 16 weeks of age or older. The sera were preserved at —20°C.

Determination of thyroglobulin antibodies

The test was performed in plastic V-shaped-bottom microtitre trays (Cooks Engineering Company, Alexandria, Virginia, USA). Prior to testing, the sera were inactivated at 56°C for 30 min., absorbed with washed erythrocytes for 1 hr. at room temperature and for 1 hr. at 4°C to absorb agglutinins against the cells to be used in the test.

To 0.05 ml of double fold serum dilutions made in phosphate-buffered saline (pH 7.2) in the plastic tray, the same volume of coated cells was added. The reading of the settling pattern of cells was made after keeping the plastic tray for 2 hrs. at room temperature. The extent of agglutination was graded from 1 to 3, and grades 2 and 3 were considered positive. The titres were expressed as reciprocals of serum dilutions.

Forty-five OS sera were tested in 4 test series using human erythrocytes, and in each series 1 control was included. The titres of the control sera were the same in different series of the test in which they were included. Out of the first-mentioned, 8 were subsequently tested in 2 series using chicken erythrocytes. Serum no. 10 was included in both series and gave the same titre in both.

Inhibition test

Chicken liver extract for the inhibition test was prepared in the same way as the chicken thyroid extract described above. Prior to testing in the inhibition experiment, the chicken tissue extracts were kept in boiling water bath for 2 min. and clarified by centrifugation as described by *Witebsky et al.*

In the inhibition test 0.05 ml of no. 3 serum at constant dilution 1:4 was added to 0.05 ml of double fold serial dilutions of chicken thyroid extract and of chicken liver extract, respectively, in the plastic tray. The serum extract mixture was incubated at 37°C for 1 hr.; then 0.05 ml of coated human cell suspension was added. The reading of the settling pattern of cells was taken after 2 hrs. incubation at room temperature.

Tanned agglutination test

In order to compare the tanned agglutination method with the present method, 2 pools, A and B, were prepared from various OS sera and ultracentrifuged in order to remove the lipids. Both pooled sera were tested using human erythrocytes according to the present method and by the TA method as described by *Witebsky et al.*

RESULTS

The sera of 45 OS chickens, tested by the present method and using human erythrocytes and chromic chloride as coupling reagent, gave the results stated in Table 1. Thirty-three sera were positive in titres ranging from 4 to 1024. The settling pattern was good. The test was easy to read. No difference was observed in the settling pattern between the tests with sera containing lipids and the tests with control 2 and pools A and B, from which the lipids were removed.

Table 1. Titration of OS chicken sera for thyroglobulin antibodies by the passive hemagglutination method using chromic chloride as a coupling reagent. Both human and chicken erythrocytes were employed.

No. of chicken	Titre, by using erythrocytes		No. of chicken	Titre, by using erythrocytes	
	human	chicken		human	chicken
1	4	—*	25	0	—
2	4	—	26	0	—
3	128	—	27	16	8
4	8	—	28	8	—
5	16	—	29	0	—
6	4	—	30	8	—
7	0	—	31	8	8
8	0	—	32	64	16
9	1024	—	33	32	—
10	64	32	34	0	—
11	16	—	35	512	—
12	16	—	36	32	—
13	64	64	37	0	0
14	32	—	38	32	8
15	16	—	39	0	—
16	32	—	40	16	4
17	64	—	41	0	—
18	4	—	42	0	—
19	32	—	43	0	—
20	0	—	44	64	—
21	4	—	45	4	—
22	64	—	control 1	32	—
23	16	—	control 2	128	—
24	8	—			

* not tested.

Sera nos. 1 to 5 were taken from chickens 16 weeks of age and nos. 6 to 45 from chickens 18 weeks of age.

In the inhibition test performed with serum no. 3 chicken thyroid extract up to dilution 512 inhibited the agglutination of chicken thyroid extract-coated cells, whereas chicken liver extract showed no inhibition.

The titres of sera found when chicken erythrocytes were used instead of human erythrocytes tended to be slightly lower in the conditions of our test (Table 1).

The comparison of the sensitivity of the present method with that of the TA test gave the following results: Using human cells, the pooled sera A and B tested by the present method gave titres 64 and 32, while the tanned agglutination method gave the titres 16 and 8, respectively. Control 2 showed titres 128 and 32 respectively.

DISCUSSION

The result of the inhibition test supports the idea that what has been measured are antibodies against components or products of the thyroid glands. Circulating antibodies to thyroglobulin have been demonstrated in the sera of OS chicken (*Cole et al.* 1968). By the immunofluorescence technique the suggestion has been gained that antibodies are formed against thyroglobulin only; no antibodies, directed against antigenic components of the thyroid epithelium of OS chickens, were demonstrated (*Wick et al.* 1970). Thus it can be conjectured that what has been measured in the present test is exclusively antibodies against chicken thyroglobulin.

Aho et al. (1971) reported that for the determination of thyroglobulin antibodies in a human population the thyroglobulin test using chromic chloride as a coupling reagent is fairly sensitive as compared to the previous TA test. The results obtained with the pooled OS sera A and B and with the control serum 2 suggest a similar higher sensitivity of the present method, compared to the TA test, when chicken thyroglobulin antibodies are measured.

As further advantages of the present method the following could be listed: In the TA test the sera of OS chickens do not produce the characteristic infolding pattern (*Witebsky et al.* 1969), whereas in the present method the coated cells give a good settling pattern easy to read. OS sera are very rich in fatty substances, and it is important to ultracentrifuge all sera prior to

testing them in the TA test, as otherwise the hemagglutination patterns may be altered (*Witebsky et al.*). In the present method OS sera can be tested without ultracentrifugation. This point is naturally of practical importance. Moreover, the present method is easier to perform, it requires less antigen, and it is less time-consuming than the previous tanned agglutination test. The lower reactivity of chicken erythrocytes as compared to human erythrocytes in our test conditions is not easy to understand.

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SAMMANFATTNING

Bestämmandet av immunkroppar mot thyroglobulin i sera från höns av Obese-stam med användning av kromklorid som kopplingsagens.

Kromklorid användes för att fästa höns-sköldkörtelextrakt på humana och på hönserythrocyter. Med så preparerade celler bestämdes immunkroppar mot thyroglobulin i sera från höns av Obese-stam. Ultracentrifugering av sera för försöket var onödig. Metoden är känsligare och snabbare och antigenbehovet mindre jämfört med den tidigare tekniken där tanninbehandlade blodkroppar användes.

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Reprints may be requested from: Jaakko Tuomi, Department of Microbiology and Epizootology, College of Veterinary Medicine, Hämeentie 57, SF-00550 Helsinki 55, Finland.