comprising the usual placental sign; and 3) a loss of weight by the mother after the 10th day of pregnancy.

Malformations involving the eye, cardiovascular system, and genito-urinary tract were found in the fetuses or newborns from all but one of the pregnancies from which intact offspring were obtained after the 14th day of gestation.

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Beef Erythrocyte Extracts Reacting with Hemagglutinins in Infectious Mononucleosis and in Horse Serum Sickness. (17544)

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In previous publications(1,2) it was demonstrated that 2 serologically active substances can be isolated from the stroma of beef erythrocytes; one reacts with the hemagglutinins that are formed in infectious mononucleosis, the other, with those formed in horse serum sickness. The stroma of beef erythrocytes was first exhaustively extracted with acetone and then with 100% ethanol at room temperature, thus removing serologically inactive substances to a large extent. In order to isolate the specific reactants the stroma residue was then extracted with boiling 100% ethanol, and subsequently, with boiling 80% ethanol. As can be seen from Table I, the 80% ethanol extract yields the so-called mononucleosis antigen (M.A.) and the extract obtained with boiling 100% ethanol, the so-called serum sickness antigen (S.S.A.). Complete and distinct separation of the two serologically active substances could not be accomplished. The fractions do not correspond to pure haptens. However, on the basis of serological activity it could be assumed that the individual fractions were only 2-6% contaminated by the heterogenic fractions. Since the individual fractions are serologically highly active it was thought probable that they might demonstrate significant chemical differences which might point to the nature of the individual haptens. On the basis of previous investigations the nature of these haptens was disclosed only to the extent that they were found to be thermostable, not digestible by trypsin and pepsin, and did not give reactions characteristic for proteins. This report deals with the chemical analysis of the serologically active fractions of the beef erythrocytes. In addition, the glucose and glucosamine content after hydrolysis is presented.

Methods. 1. The C–, H– and N– determinations were done according to Pregl's method of quantitative elementary microanalysis.

2. Phosphorus was determined as ammoniumphosphomolybdate.

3. The reducing power was determined according to the Hagedorn-Jensen method and calculated for the glucose equivalent. The fractions were hydrolyzed at 100°C with 2 N HCl for 2 hours, then neutralized.

4. The glucosamine determination was done according to the method of Boyer and Fuerth as modified by Brunius.(3) 25-75 mg of dry substance were suspended in 7.5 ml of 4 N HCl and diluted with distilled water to 10 ml. This was hydrolyzed at 100°C in a sealed test tube for 5 hours. After filtration, whenever necessary, color clarification was effected by charcoal adsorption. This solution was carried to dryness over KOH and redissolved in 2 ml of distilled water. This, after

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1. Schwarzweiss, H., and Tomcsik, J., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 558.

2. Tomcsik, J., and Schwarzweiss, H., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 562.

3. Brunius, F. E., *Chemical Studies on the True Forssman Hapten, etc.*, A. B. Fahlcrantz Boktryckeri, Stockholm, 1936, p. 141-144.

TABLE I.
Extracts of Beef Stroma and Their Inhibition Titer Toward Sheep Cell Agglutinins.

Extraction	Extracted substance in %	Inhibition titer toward sheep hemagglutinins in	
		Inf. mononucl.	Serum sickness
Acetone, room temp.	8.6	0	0
Ethanol, 100% room temp.	12.6	250	16,000
" 100% boiling	0.7	16.000	*250,000*
" 80% "	5.1	*1,000,000*	16,000

TABLE II.
Chemical Analysis of the Ethanol Extracts of Beef Stroma.

% of	Ethanol. 100% room temp.	Ethanol, 100% boiling	Ethanol, 80% boiling
C	68.13	67.16	56.61
H	11.88	10.54	7.74
N	3.04	3.47	8.45
P	0.35	0.37	2.44
Reducing sugar	2.7	8.31	17.4
Glucosamine	1.76	4.03	8.56

addition of acetylacetone and N Na_2CO_3, was heated on the water bath and acidified with acetic acid; Ehrlich's reagent was added, air was blown through, and the volume was diluted to 10 ml with glacial acetic acid. The intensity of the red color obtained was determined with a Klett-Summerson photoelectric colorimeter. The calibration curve within the range of 0.2-1.4 mg of glucosamine, was approximately a straight line. The glucosamine content of the unknown solution was interpolated according to this calibration curve.

Results. The serological activities of the various beef stroma fractions are shown in Table I. The results of their chemical analyses (average values of several determinations) are given in Table II.

The analytical values of the S.S.A. containing substance, (extracted with boiling 100% ethanol), shows a higher content of reducing sugar and glucosamine than the slightly active fraction obtained with 100% ethanol at room temperature. Significantly different analytical values were found in the M.A. containing substance which had been extracted with 80% ethanol; here the C– and H– content were lower, the content of N– and P–, as well as that of the reducing sugar and of glucosamine content, were considerably higher. After further purification(1) of the M.A. extract, both its serological activity and reducing properties were somewhat increased (reducing sugar 19.4%). The inactive acetone extract was tested only for reducing substances after the Hagedorn-Jensen method (reducing sugar 0.23%).

The chemical nature of the serologically active M.A. and S.S.A. containing fractions of the beef erythrocytes cannot be determined because of the lack of purity of the preparations. However, it may be inferred that both substances, especially the so-called mononucleosis antigen, belong to the same group of substances as the human A substance and the Forssman antigen, and that glucose or glucosamine play an important role in the hapten structure.

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