Serum Protein and Glycoprotein Changes in Chickens Bearing the Rous Sarcoma[1]

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Rous (1910) reported the transmission of a tumor from one chicken to another by an agent separable from the tumor cells. Electrophoretic-separable components of serum from animals bearing transplantable tumors have been studied by Shetlar, *et al.* (1950) and Wall and Schlumberger (1957). However, similar studies of the effect of the Rous sarcoma have not been reported. This is a preliminary report on the serum protein and glycoprotein changes in chickens infected with Rous sarcoma virus.

MATERIALS AND METHODS

Twelve, 4-to 6-week-old, Honegger White Leghorn chickens of both sexes were divided into 2 equal groups of control and experimental animals. The control and experimental animals were maintained in separate cages under similar environmental conditions. After a control serum sample was taken from each animal in the 2 groups, the experimental chicks were inoculated in the wing web area with Rous sarcoma virus. Subsequent blood samples were obtained from each chick, by cardiac puncture, at 2-, 4- and 5-week intervals after the experiment was begun. Because of the small amount of blood that could be obtained from each chick at one time without endangering its life, the serum samples from each group were pooled for chemical and electrophoretic studies. At the end of the fifth week the experiment was concluded. Weights and micro-hematocrits of each animal were measured immediately following each cardiac puncture.

Sera were subjected to paper electrophoresis studies using a Spinco Model R. paper electrophoresis cell. A current of 5 millamperes per cell was applied for 16 hours. Twenty microliters of serum were applied to strips for protein and 50 microliters to strips for glycoprotein determinations. Following a run of 16 hours, the strips were dried immediately in an oven at 110° C. for 30 minutes. The protein strips were stained with bromphenol blue (Spinco Model R. Paper Electrophoresis System Instruction Manual, 1957) and the glycoprotein strips were stained by the periodic-acid-Schiff (P.A.S.) method as described by Shetlar, *et al.* (1956).

The amount of dye bound by each of the various fractions was determined by direct optical scanning of the strips with the Spinco Model RB Analytrol, with a B-5 cam. The protein strips were placed in an atmosphere of ammonium hydroxide for 15 minutes before being scanned with a No. 500 light filter. The glycoprotein strips stained with P.A.S., were scanned with a No. 550 light filter.

Total serum protein was established by the biuret method of Weichselbaum (1946) and total serum glycoprotein by the method of Shetlar, *et al.* (1948).

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RESULTS

In Table I are recorded the changes in weight and microhematocrit that occurred during the experiment. In regard to weight, one can note that, although both groups gained weight as the experiment progressed, the experimental chickens inoculated with Rous sarcoma virus gained much less. While the microhematocrit changes were not as definitive, it appears that there is a decrease of the microhematocrit in the experimental animals. These data are consistent with those found in tumor-bearing mammals where anemia is a common finding.

TABLE I WEIGHT GAINS AND MICROHEMATOCRIT CHANGES OF TUMOR-BEARING AND CONTROL CHICKENS

	GROUP	
	Tumor	Control
Weight Gain, gms.	420	690
Hematocrit*	34-29	38-36

*First figure intial hematocrit. Second figure 37 days after infection.

TABLE II

DAYS	GLYCOPROTEIN*	PROTEIN	RATIO
CHICKENS WITH ROUS SARCOMA			
0	52	2.94	1.77
37	60	2.93	2.05
RATS WITH WALKER 256 TUMOR**			
0	155	5.7	2.71
35	193	4.3	4.49

* Expressed as mg. of bound hexose/100 ml. of serum.

** Shetlar, et al. Cancer Res. *10,* 445, 1950

TABLE III
SERUM PROTEIN FRACTIONS OF CHICKENS WITH AND WITHOUT ROUS SARCOMA

GROUP	DAYS	SERUM FRACTIONS*				
		I	II	II-2	III	IV
Control	0	2.7	51.8	-	19.8	25.7
Tumor	0	2.2	45.8	-	17.6	34.4
Control	37	2.2	51.7	-	18.5	27.5
Tumor	37	1.5	21.0	10.5	19.0	38.0

*Expressed as a percentage of the total serum protein.

Table II contains a summary of the total serum protein and glycoprotein from the beginning to the termination of the experiment. For comparison, similar data from earlier work by Shetlar, *et al.* (1950) on rats bearing the Walker 256 tumor are included. Although the differences in the current study are not so striking, the changes in the 2 species are in the same direction, i.e., an increase in carbohydrate-rich protein as the tumor increases in size. More striking differences in the present study might have been observed if they had been continued until a decrease in serum protein had occurred.

As can be noted in Fig. 1, four main serum fractions, that were clearly recognizable at all times, were found on the protein strips. These fractions were designated as Numbers I, II, III and IV. In comparison to human serum run in the same cell at the same time, fraction I appeared to be a pre-albumin fraction, having no counterpart in the human strip. Fraction II corresponded in mobility to the human albumin, fraction III to alpha globulin of human, and fraction IV to the beta and fast gamma globulin fractions of human sera. There did not appear to be a definite slow moving part of gamma globulin as is found in humans. The normal glycoprotein fractions were similar except that no fraction I or pre-albumin fraction was noted. In later stages of the tumor-bearing chickens, a fifth fraction was noted between fractions II and III, which has been designated II-2. This fraction was present on both protein- and glycoprotein-stained strips, indicating that it contains bound carbohydrate.

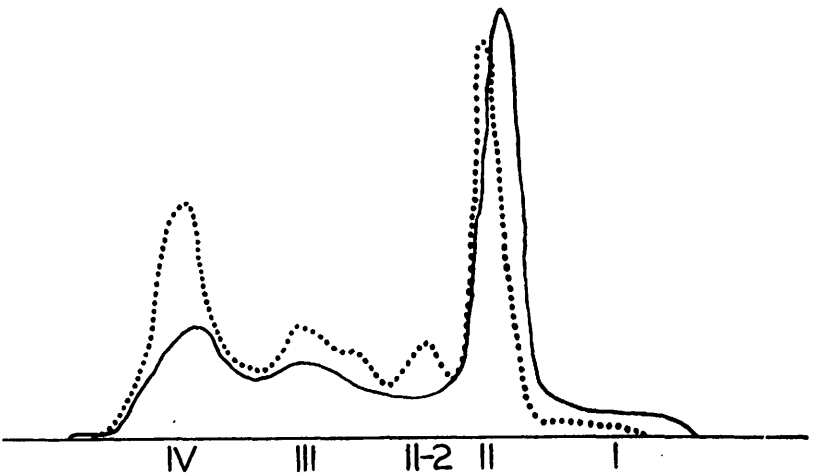

Figure 1. Densitometer patterns of paper electrophoretic patterns of the sera of chickens with and without Rous sarcoma. Solid line is from control chickens of the same age as those infected with the Rous sarcoma virus (dotted line). Note the appearance of a new fraction (II-2) in the tumor group.

Protein changes in various serum fractions after growth of the tumor can be seen in Table III. As far as the control groups were concerned, no consistent changes were found in the proteins during the period of study. On the other hand, definite changes were noted in regard to the tumor-bearing animals. There appeared to be decreases in fractions I and II. The decreases were balanced by the appearance of fraction II-2. Fraction III appeared to remain about the same while there was an increase in fraction IV as the experiment proceeded.

SUMMARY

A preliminary study of the changes which occur in the serum protein and glycoprotein of chickens bearing the Rous sarcoma was made. By paper electrophoresis technics, 4 protein fractions were noted in the serum of chickens. Three of these, stained by the periodic-acid-Schiff technic, indicate that they contain appreciable amounts of bound carbohydrates. A fifth protein fraction, which also stained in the periodic-acid-Schiff technic, was noted in the sera of chickens bearing large tumors. A trend toward a higher bound carbohydrate (glycoprotein) to protein ratio was

noted in the tumor-bearing birds. A failure of the tumor-bearing chickens to gain weight at the usual rate and the development of a slight anemia were noted.

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