Zamis (*7*). These results contrast strikingly with reports by the workers with rats (*1, 2*) that 80–95% of their animals were infected.

So far as mice are concerned, therefore, otitis media is not necessary for susceptibility to audiogenic seizures. Further, the very low incidence of the disease in mouse colonies makes it negligible as a complication in studies on auditory reactions.

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Immunochemical Changes in Chicken Serum During Development of Rous Sarcoma[1]

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It is well known (*1–9*) that during the growth and maturation of the individual changes occur in the serum, manifested by an increase in globulin and by the appearance of various antibodies for cells of foreign species and multiple infectious agents. In the chicken one finds antibodies against tumor viruses, e.g., the Rous sarcoma (*3*), and also, as recently found in this laboratory, against *Proteus*, a bacterium that thrives in autolyzed extracts of this same sarcoma.

Furthermore, it is recognized (*1*) that, as a common denominator to the development of natural or experimentally induced antibodies in chickens, a factor appears in the globulin fraction of the serum which is endowed with the property of flocculating, in the cold, saline or alcoholic extracts of many tissues from many species of animals.

On the other hand, it has been found by several workers (*4–6*) that chickens bearing the Rous sarcoma or a lymphocytoma show a hypoproteinemia depending not on changes in the plasma volume (*6*) but rather, in the case of the lymphocytoma, on a reduction of albumin and occasionally of globulin.

In our studies on the immunochemical changes in the serum during malignancy, we focused our attention on the naturally present antibodies as well as on the factor flocculating tissue extracts, and it was soon apparent that in chickens bearing the Rous sarcoma there was a diminution in some of these immune bodies. Although this decrease was quite evident with the globulin factor flocculating tissue extracts in our test (mouse liver), it was most clear-cut in an agglutination reaction with *Proteus*. We paralleled these studies with an analysis of serum protein and have compared the results of both methods in the following note.[3]

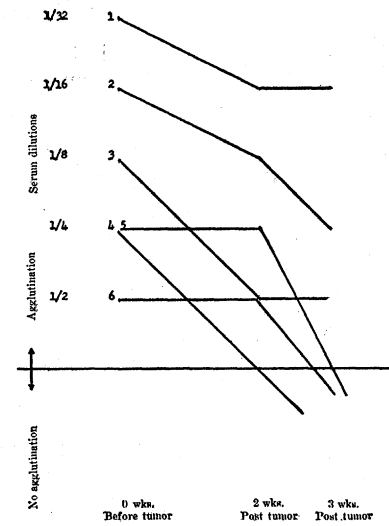

FIG. 1. Decline of *Proteus agglutinin* in chicken serum during development of Rous sarcoma.

Proteus agglutinin can be readily detected by combining serum with a live culture, vaccine, or O antigen of the *Proteus vulgaris* or *OX19* strains, both of which are equally suitable.[4] The organisms were grown in broth for 24 hr and then transplanted to agar for the same length of time. The agar growth was suspended in physiological saline, to which we added 30% by volume of alcohol and incubated the mixture overnight at 37° C. At this stage the bacilli were usually dead and could be centrifuged to obtain a precipitate which was resuspended in 7 equal volumes of saline. Before testing, this initial stock solution was further diluted 10–15 times with saline, depending upon trial tests necessary to determine the greatest dilution that would give agglutination with normal central sera. The actual test was done in small test tubes (1.2 cm × 10.1 cm) protected with cork stoppers. To each tube containing 0.1 ml of serum, inactivated or not, undiluted or in dilution, we added 0.1 ml of antigen and, after incubating at room temperature, read the tubes with the aid of a binocular microscope (10 × and 23 ×) at several time intervals over a 2-hr period. Similarly, the presence of the tissue flocculating factor in chicken serum was indicated by flocculation with a 5% saline extract of mouse liver kept at 0° C for 24 hr.

Both determinations indicated a high incidence of

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[2] National Cancer Institute special research fellow.

[3] While this paper was in press, the Lankenau Hospital reported similar results with mice (*12*).

[4] The strains were obtained from the American Type Culture Collection. In the earliest stages of our work, however, we used *Proteus* which was isolated and identified through the kindness of Leanor Haley, from a contaminated extract of chicken sarcoma.

these immune bodies in normal adult chickens which develop some time after one month of age and which diminish so rapidly in the wake of a Rous sarcoma that, in many cases, they could scarcely be detected at the end of 2–3 weeks. On every sample of serum taken before and after tumor growth, titrations were done simultaneously with the same antigen preparation. Although the titers of *Proteus agglutinin* were rather low, a significant decline was constantly observed in the several dozen birds followed, and we have selected 6 representative sera for the accompanying chart (Fig. 1). In these experiments with rapidly growing tumor the chickens were dead 4–5 weeks after tumor transplant. In all, we used more than 60 birds, some White Leghorn, but chiefly Plymouth Rock.

Concurrent with our investigation of antibody titer, we determined serum proteins by the biuret method of Gornall *et al.* (*10*), spot-checking the results against Kjeldahl analysis. Our fractionations were done with either the ammonium sulfate or sodium sulfate (*11*) procedure. All these determinations indicated that serum protein undergoes considerable change in the development of a normal chicken. Young chicks have less protein per ml of serum when compared to the increase in adult fowl where the augmented globulin fraction is mainly responsible. This difference can be expressed by the albumin globulin ratio, which is greater than 1.0 in young chicks and completely reversed in adult birds. In fact, our fractionation with sodium sulfate gave an A/G ratio of 0.35 for normal adult chickens, which closely approximates the 0.37 determined by electrophoretic studies (*6*). This value may then be compared to the 0.86 ratio of one-month-old chicks and the 1.08 ratio we determined in 10-day-old chicks (Table 1).

In adult chickens with a rapidly growing Rous sarcoma we found a hypoproteinemia of varying degree, some showing only a very slight drop in total protein. Fractionation of the sera of these birds revealed a moderate reduction in albumin, whereas the globulin, more specifically euglobulin fraction, changed significantly. On the average, the total protein reduction in tumor-bearing birds was about 22%, whereas the average reduction of albumin was only 14%. A hypoglobulinemia resulted from an average drop in total globulin equal to 22%, being as much as 40% in some cases. Furthermore, we found a reduction in the euglobulin fraction even in cases where there was an insignificant fall in the total serum protein. This is of particular interest, since we were able to locate the *Proteus* antibody and the liver extract flocculating factor in the euglobulin fraction and have recovered them almost quantitatively by fractionation with ammonium sulfate (21.6% $(NH_4)_2SO_4$ on dry weight basis). Although our titration of the immune bodies in different concentrations of protein (i.e., euglobulin) reveals that they decrease in chickens with massive tumor faster than does the diminishing euglobulin, nevertheless this reduction of euglobulin in birds with fast-growing Rous sarcoma is comparable to the diminished γ-globulin of late human cancer (*14*).

TABLE 1

SERUM PROTEIN IN CHICKENS OF DIFFERENT AGES (mg/ml)

Determination	Adult—average value and deviation	Combined bleedings (1 month)	Combined bleedings (10 days)
Total protein*	55.7 ± 6.3	37.3	34.9
Euglobulin	29.8 ± 5.5	—	—
Pseudoglobulin	10.7 ± 2.1	—	—
Total globulin	42.2 ± 5.8	20.0	16.8
Albumin	14.1 ± 3.1	17.3	18.1
A/G	0.35 ± 0.2	0.86	1.08

* Our values for total protein correspond to the analysis of Herman (*13*).

It should be noted that similar investigations made on normal birds, cohabitating with the diseased chickens and bled at the same intervals, indicated no comparable change. These normal chickens evidenced a small gain in weight, whereas the tumor-bearing birds failed to gain or even showed a slight loss. We consider these variations insignificant, however, in comparison to the described changes in the pattern of serum protein.

Thus with chickens bearing a rapidly developing Rous sarcoma, we note a decline in serum globulin (i.e., euglobulin) which is manifested by the suppression of natural immune bodies as indicated by the reduction of *Proteus agglutinin* and the flocculating factor from tissue extracts. Since this fall in antibody titer preceded any diminution of the globulin fraction, it appears to be an indication of a qualitative change in globulin, and it seems to us that such a method of nonspecific antibody titration could afford a sensitive index of early change in serum globulin.

Phenomena in many ways comparable to those described have been found in human sera and have been reported in another article (*15*). Furthermore, experiments concerned with the effect of chemical carcinogens on the serum globulin are also under way.

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