9. Schweinburg, F. B., unpublished observations.
10. Schweinburg, F. B., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 589.
11. Greisman, S. E., *J. Exp. Med.*, 1960, v112, 257.

Received November 18, 1960. P.S.E.B.M., 1961, v106.

Utilization of Serum a_1-Globulin and Glycoprotein by *Diplococcus pneumoniae*.* (26350)

YU-TEH LI AND M. R. SHETLAR
Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City

Changes of the serum protein fractions and of the bound carbohydrate (glycoproteins) of these fractions are known to occur in many pathological conditions. However, little is known about the metabolic alterations which lead to these changes, about the normal synthesis and destruction of glycoproteins in the animal body. Information is also lacking concerning destruction of the blood glycoproteins by micro organisms. A preliminary survey in this laboratory involving a number of micro organisms revealed a surprising resistance of serum glycoproteins to bacterial destruction. Data reported by Friedemann and Sutliff(1), however, indicate that the pneumococcus may utilize the protein bound carbohydrate of sera from individuals with certain pathological conditions.

In recent experiments, a strain of *Diplococcus pneumoniae* was utilized which had been isolated from a patient with pulmonary pneumonia at University Hospital, Oklahoma City, and maintained in frozen stock.† Five ml of sterile serum was transferred to a 50 ml sterile Erlenmeyer flask and inoculated with one loopful of 6-hour, rapidly growing culture of the organism. The inoculated serum sample was incubated at 37°C for 20 hours. A control serum sample, similar in all respects except uninoculated, was incubated for the same time in the same way. After incubation, the bacterial cells were removed by centrifugation. Determinations of protein were made on the supernatant by the biuret method; glycoprotein hexose was determined by the method of Shetlar *et al.*(2). Two samples of bovine sera, 2 rat samples, and 8 human samples (4 normal, 2 cancer, 2 rheumatoid arthritis) were investigated. The inoculated bovine samples decreased from 151 to 130 mg per 100 ml in glycoprotein content, and from 7.13% to 6.46% in protein content. Similar changes were noted in rat sera. Results of the human studies are summarized in Table I. In every instance, samples incubated with the organism decreased in both protein and glycoprotein content. When the normal human samples were considered, the difference between serum glycoprotein contents of inoculated and control groups was statistically significant at the 1% level; the decrease of the protein content was also significant at the 1% level.

These data indicate the preferential destruction of carbohydrate-rich protein by the micro organism. This is confirmed by paper electrophoretic studies of protein and glycoprotein of the sera by methods previously described(3). Results of a typical study of serum from a cancer patient are shown in Fig. 1. Results of a_1-glycoprotein determined by paper electrophoresis are given in Table I. It appears from the electrophoretic pattern and from the results in Table I, that the micro organism preferentially utilizes a_1-glycoprotein. Similar results were found in paper electrophoretic investigations of all human samples and of rat and bovine sera. The decrease of a_1-globulin averaged 85% (ranging from 50-100%);

* This study supported by a grant from U.S.P.H.S. (Nat. Cancer Inst.).

† The authors are greatly indebted to Dr. Florene Kelly and Mr. Billy West, Dept of Microbiology, for the culture and for advice on bacteriological technics.

TABLE I. Protein and Glycoprotein Changes of Human Sera after Incubation with *Diplococcus pneumoniae*.

Condition	Total protein, g/100 ml		Total glycoprotein, mg hexose/100 ml		α_1-Globulin glycoprotein, mg hexose/100 ml	
	Organism*	Control	Organism	Control	Organism	Control
Normal (4)	6.64 ±.07†	6.93 ±.08	99 ± 3.1†	117 ± 3.5	0	19
Carcinomatosis	4.75	5.04	134	174	12	62
Breast cancer	7.12	7.62	152	176	12	38
Rheumatoid arthritis	6.10	6.66	129	180	0	45
Idem	5.87	6.19	158	182	9	32

* Serum incubated with organism for 20 hr at 37.5°C. Control incubated in same way at same time.

† Significantly lower than control value at 1% level. Values following ± signs are stand. errors of mean.

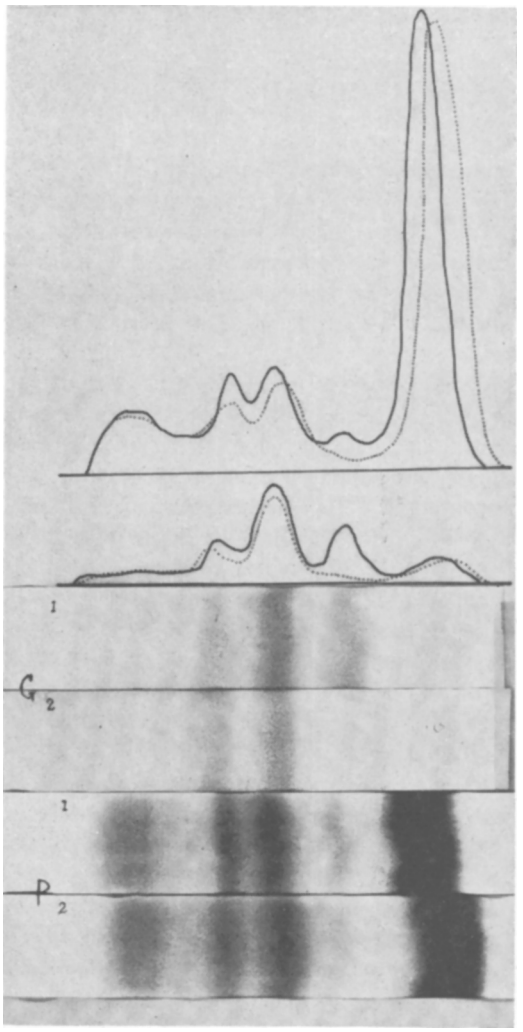

FIG. 1. Alterations of electrophoretic patterns of human serum after incubation with *Diplococcus pneumoniae*. Lower 2 strips (marked "P") are stained for protein with bromphenol blue. No. 1 is of control (incubated without organism); No. 2 is strip of the sample after incubation with microorganism. Note decrease of α_1-globulin fraction (second band from right). Upper strips (marked "G") are corresponding strips stained by the periodic-acid-Schiff procedure for glycoprotein. Again the α_1 fraction is nearly absent in sample incubated with the organism (No. 2). Above both strips are densitometer tracings of these patterns. Protein tracings (from "P") are at top; immediately below are glycoprotein tracings. In both cases, control tracing shown by solid line; those from samples incubated with the organism shown as dotted lines. Note decrease of α_1-globulin peaks in each case.

when considered in absolute terms (milligrams per 100 ml of serum), the decrease of α_1-globulin hexose was approximately equal to the loss of total glycoprotein hexose. No other fraction was appreciably affected.

Further studies are required to determine whether the α_1-globulins (or other globulins) from the serum of patients with certain diseases are used more readily by pneumococcus than those from normal sera. It seems likely, however, that the increased lactic acid production by the pneumococcus from sera of patients with pathological conditions as previously described(1) may be due to increased α_1-globulin in these sera. Elevations of the α_1-globulins and the glycoproteins of this fraction are known to occur in these diseases.

These studies are of interest in connection with the recent report of Kent and Gey(4) which indicated a preferential utilization of α_2-globulins by rat tumor cells. Of wider importance is the possibility that enzyme systems may be isolated from the pneumococcus which will be useful in degradation studies of the glycoproteins.

Summary. When serum samples were in-

cubated with a rapidly growing strain of *Diplococcus pneumoniae,* striking decreases of serum glycoprotein and consistent decreases of protein resulted. Paper electrophoretic studies indicated that these changes were largely restricted to the a_1-globulin fraction. It would appear that this strain of *Diplococcus pneumoniae* preferentially uses glycoproteins found in the a_1-globulin fraction of mammalian serum.

1. Friedmann, T. E., Sutliff, W. D., *Science,* 1939, v90, 335.
2. Shetlar, M. R., Foster, J. V., Everett, M. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 125.
3. Shetlar, M. R., Cahill, C., Stidworthy, G., Shetlar, C. L., *ibid.,* 1956, v93, 44.
4. Kent, H. N., Gey, G. O., *Science,* 1960, v131, 666.

Received November 23, 1960. P.S.E.B.M., 1961, v106.

Endemic Toxoplasmosis in Isolated Swine and Cattle Herds and Its Relationship to a Human Population.* (26351)

NANCY MITCHELL WENDE AND ROBERT B. DIENST (Introduced by Knowlton Hall)
Department of Medical Microbiology and Public Health, Medical College of Georgia, Augusta

Serological findings have shown that toxoplasmosis occurs as an attenuated infection in man and many warm blooded animals(1, 2,3). Rarely does overt disease occur with *T. gondii* when one considers the high incidence of infection. The means of natural transmission remain unknown(4). The present study presents additional information for natural transmission from animal to animal and from animal to man in an isolated environment.

In a recent study of 703 patients at the Georgia Training School for Mental Defectives,† Gracewood, Ga., serologic procedures had pinpointed a high incidence of attenuated infection in 2 of 12 cottages. In these 2 cottages 51% of 86 patients were positive to the toxoplasmin skin test as compared to 15.4% of 617 patients in other cottages. The only related identifiable factor which differentiated these 2 cottages was that they housed the patients who assisted in the farm work and in the care of the farm animals. Each of the 12 cottages had a relatively stable population which slept in that cottage and ate together to form a "family group." A central kitchen prepared the food for all cottages; milk was purchased from an outside dairy. There was a central water supply.

Investigation of infection in the 2 large animal herds maintained at Gracewood revealed the high incidence shown in Table I.

The farming program at Gracewood was under the direction of a graduate agriculture engineer and all animals were under the medical supervision of a licensed veterinarian. No epidemic or unusual disease of acute or subacute character had occurred among the animals for the past 9 years. The herds investigated served as a source of beef and pork for the Training School; all meat passed U. S. Government standards. The swine herd had been kept for over 12 years in a pasture adjacent to the slaughter house. The beef cattle were pastured with the swine until 1957 when they were removed to new pastures 5 miles distant; however, cattle were still occasionally pastured with the swine at intervals to control the forage crops. The pasture was plowed twice yearly to minimize parasite infestation. All garbage collected from the Training School was heated to 212°F for a minimum of 30 minutes before being fed to swine. Warfarin was placed out routinely for rodent control.

Isolation of T. gondii. To substantiate serological incidence of infection in the swine herd, isolation attempts were made on the brain tissue of 14 randomly selected hogs that were slaughtered for table consumption.

* This investigation was supported by grant from Nat. Inst. Health.

† Appreciation is expressed for the cooperation of the Gracewood Training School administrative staff.