and attains a maximal concentration of approximately 10,000 G.P.U. per g of ovarian tissue by the time the fetuses are 5 or 6 inches in length. It is rather surprising, in view of this high concentration in the ovaries, to find only 2 G.P.U. of the hormone per ml of blood serum in the pregnant animal. This is only one-fifth of the concentration found in the blood serum of pregnant rabbits.[5] However, it is 4 times the concentration found in pregnant guinea pigs[6] which indicates a wide species variation.

While the ovary must be regarded as the major source of relaxin in the pregnant sow, small amounts (0.5 to 2.5 G.P.U. per g) were also found in the placenta. Compared to the concentrations obtained in the ovary this amount is extremely small; nevertheless it is significant to note that in both the guinea pig[6] and the rabbit,[7] relaxin has been found in the placenta.

[5] Marder, S. N., and Money, W. L., *Endocrinol.*, 1944, **34**, 115.

[6] Zarrow, M. X., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 488.

[7] Zarrow, M. X., Unpublished observations.

The lack of relaxin in the ovary of the gilt and in the follicular fluid and ovary of the sow in pre-oestrus indicates that the corpora lutea are of importance in the formation of relaxative substance. The results of Albert, Money and Zarrow[1] show that relaxin is present in both the corpora lutea and the non-luteal tissue of the ovary of a pregnant sow. This may indicate that relaxin is formed in the luteal tissues and diffuses out to the surrounding area or that there is an extra-luteal site which forms relaxin under the stimulation of a corpus luteum.

Summary. No relaxin is found in the ovaries of the gilt nor of the sow during the follicular phase of the estrous cycle. There is, however, 2.5 to 5 G.P.U. of relaxin per gram of ovarian tissue present during the luteal phase of the cycle. The amount of relaxin in the ovaries increases rapidly during pregnancy and reaches a maximal concentration of approximately 10,000 G.P.U. per gram of tissue by the time the fetuses attain a length of 5 or 6 inches. The blood at mid-pregnancy contains 2 G.P.U. per ml while the placenta has 0.5 to 2.5 G.P.U. per gram.

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Electrophoretic Changes in Plasma Proteins in Patients with Pneumococcic Infections.

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Alterations of the plasma proteins in relation to successive stages of pneumococcic infections (either pneumonia or meningitis), and to important antecedent factors influencing protein metabolism, are reported in this paper. Blix,[1] who studied the plasma of pneumonia patients electrophoretically, found that the alpha-globulin concentration was more than twice normal, and gamma globulin was reduced from 76 to 64% of the total globulin concentration. Longsworth[2] confirmed these findings concerning alpha-globulin, and subsequent observers have shown that alpha-globulin generally increases in febrile reactions associated with tissue destruction, such as infectious diseases, coronary thrombosis, frac-

[1] Blix, G., *Z. f. die gesamte Exp. Med.*, 1939, **105**, 595.

[2] Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, **70**, 399.

TABLE I.
Selection of Patients with Pneumococcic Infections.

Age, yrs	Pre-existing complications	Days of illness on which observations were made	Additional protein therapy	Recovered
	Primary Meningitis.			
32	None	1,3	Serum	Yes
38	Pituitary adenoma	1,4	"	"
50	Skull fracture	1,6	"	"
60	Cirrhosis	4,7	"	No
	Primary Pneumonia			
46	None	18,25,79		Yes
28	"	4,6,14,33	Gamma globulin	"
34	"	2,4	" "	"
39	Alcoholism	21,36	Serum	"
57	"	21,24,49	"	"
46	" chr. osteomyelitis	4		No
49	"	19		Yes
70	"	10,14,31,46	Gamma globulin	"
62	" bronchiectasis	11,15,29	" "	"
42	"	8,9	" "	"
43	"	7,8,17	" "	"
79	Congestive failure	8,17,24		No
70	Emphysema	14,28		Yes
71	Heart disease	3,21		"
74	Congestive failure	5,6,32	" "	"
78	Heart disease	36		"
69	Diverticulitis	6		"
74	Cachexia senilis	4		"
65	Multiple myeloma	1,5 (months)		"

tures and burns.[3] Leutscher[4] reported greatly decreased electrophoretic albumin and increased fibrinogen concentrations in lobar pneumonia, as well as lesser increments in beta and gamma globulins. Pleural fluids similarly fractionated were found to contain the same constituents as serum, but in lower concentrations.

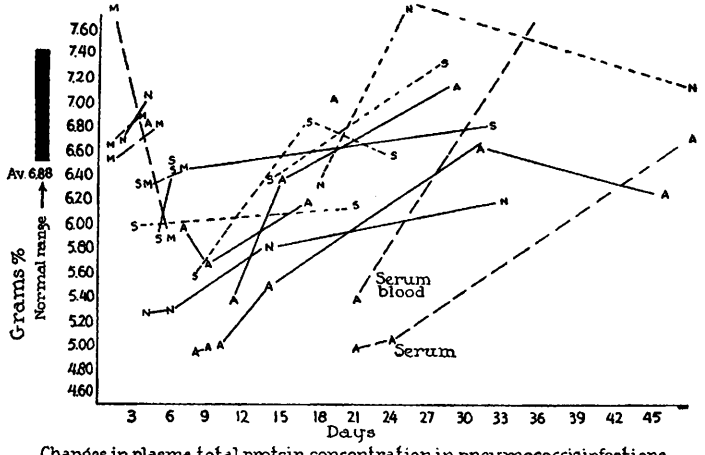
Selection of patients. During 1946, 23 patients with pneumococcic infections were selected from the Medical Clinic of the Rochester Municipal and Strong Memorial Hospitals for this study. As indicated in Table I, 4 of these patients had primary meningitis, and 19 pneumonia. In addition to routine treatment with sulfadiazine and/or penicillin, 6 were also treated with type specific rabbit antiserum because of the severity of the infection, and 7 others were given supplemental intramuscular injections of immune human gamma globulin* to investigate its effects.

Methods. The venous blood samples were drawn in dry oxalate flasks under fasting conditions to minimize lipemia, and insofar as possible with only momentary stasis. All but 3 plasma samples had a specific gravity of 1.022 to 1.026 (copper sulphate method); one from a patient with pneumonia and hepatic cirrhosis was too low to read on one day and 1.021 when repeated the following day, and 2 others were 1.029 from acutely ill dehydrated patients. Electrophoresis was carried out in the tall form of the 11 ml Tiselius cell, in diethyl-barbituric acid buffer of pH 8.5 and ionic strength of 0.1. Plasma was diluted with an equal volume of buffer and dialyzed through cellophane against 2 liters of similar buffer. Current was passed for 3 hours with a field strength of 6.8 volts per

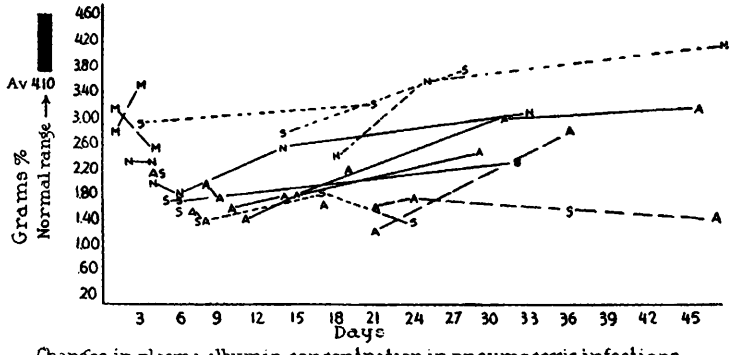
[3] Stern, K., and Reiner, M., *Yale J. Biol. and Med.*, 1946, **19**, 67.

[4] Leutscher, J. A., *J. Clin. Invest.*, 1941, **20**, 99.

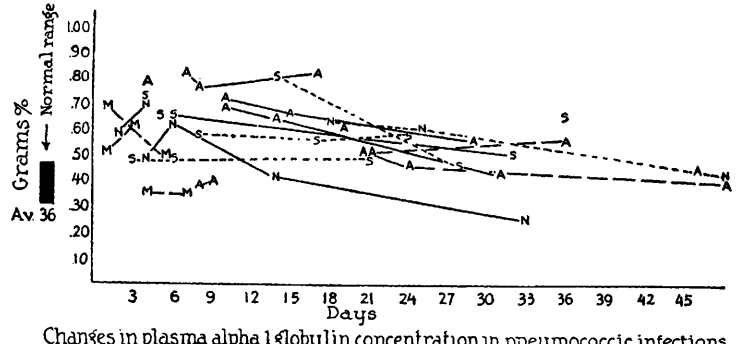
* The immune human gamma globulin was furnished by Sharpe and Dohme, Philadelphia, through the kindness of Dr. Charles A. Janeway, Boston. 50 ml of this material provided the antibody equivalent of 1200 ml of pooled normal human plasma, or not quite one-half of the estimated circulating gamma globulin in the blood.



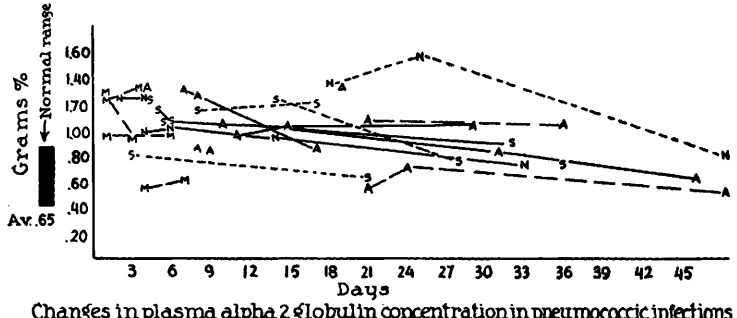
Changes in plasma total protein concentration in pneumococcic infections



Changes in plasma albumin concentration in pneumococcic infections

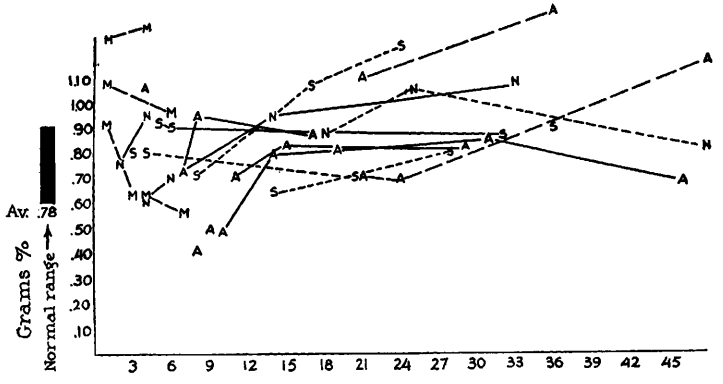


Changes in plasma alpha 1 globulin concentration in pneumococcic infections

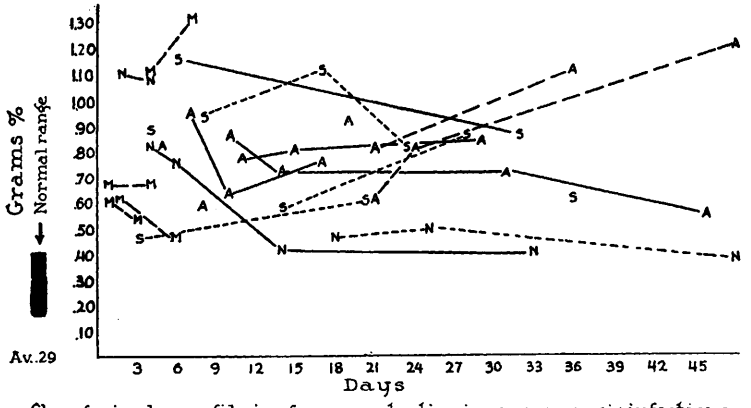


Changes in plasma alpha 2 globulin concentration in pneumococcic infections

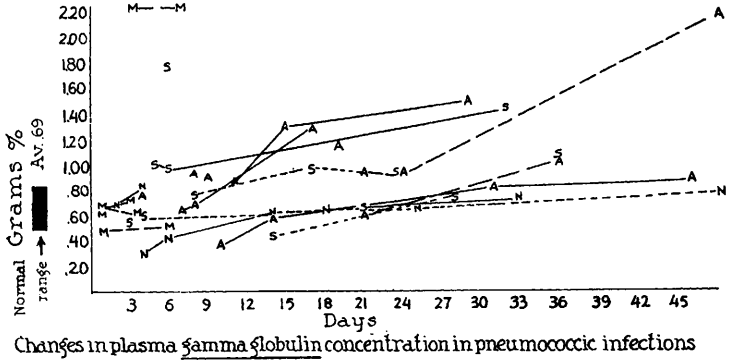
FIG. 1. Serial Changes in Plasma Protein Fractions in Pneumococcic Infections.



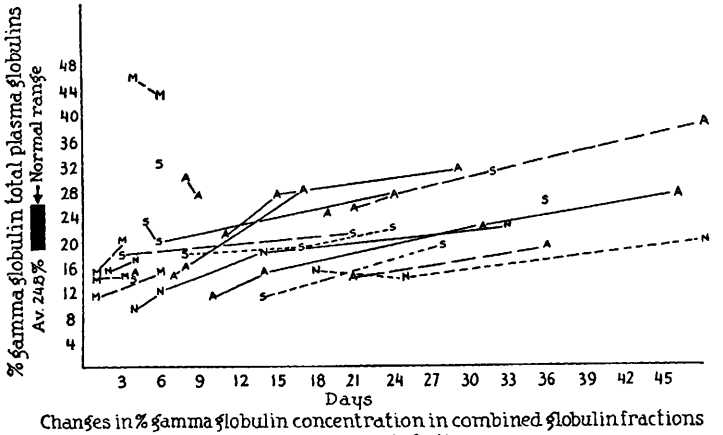
Changes in plasma beta globulin concentration in pneumococcic infections



Changes in plasma fibrinogen concentration in pneumococcic infections



Changes in plasma gamma globulin concentration in pneumococcic infections



Changes in % gamma globulin concentration in combined globulin fractions in pneumococcic infections

cm. Further details of this technic may be found in a previous paper.[5]

Results. The alterations in plasma proteins determined electrophoretically in relation to the duration of the pneumococcic infection are shown in Fig 1. In addition, the symbols indicate patient-groups: primary meningitis (M), senile (S), history of chronic alcoholism (A), and those whose previous health was normal (N). Differences in therapy are indicated by the connecting lines: small broken lines indicate routine treatment; large broken lines indicate supplemental use of antiserum; and solid lines indicate those treated with gamma globulin. For comparative purposes the range and mean values for the same fractions in 17 normal individuals are indicated in each graph.

All *total protein* levels obtained initially were somewhat depressed, except for 1 patient with meningitis and dehydration (plasma specific gravity 1.029). The blood samples from the meningitis group were obtained early in the infection and exhibited an average level of 7.07 grams%, whereas those with pneumonia were obtained later in the first and second weeks of the illness and had an average level of 5.88 grams%. There was considerable scatter, with a tendency to reach the lowest levels in the second week, and then to increase during convalescence. This parallels the observations of Rutstein, *et al.*, on plasma volume alterations in pneumonia.[6] The pneumonia patients with an alcoholic background had an average total protein concentration of 5.55 g%, whereas the non-alcoholic group averaged 6.07 g%. These values are based on samples taken from 2 to 36 days in the course of the illness, and hence are not strictly comparable. The lowest total protein of 4.9 g% was obtained from an alcoholic patient however.

The plasma *albumin* levels were low in all the initial blood samples studied. Hypoalbuminemia progressed until the second week of the infection, and all but 2 patients continued to exhibit a subnormal albumin concentration up to a month after the onset of the infection. The lowest level for non-alcoholic patients was 1.76 g%, whereas the elderly and alcoholic groups exhibited a more marked hypoalbuminemia ranging from 1.35 to 1.74 g% during the first 2 weeks. This is in contrast to the normal range of 3.7 to 4.6 g% by this method.

The *alpha-globulins* were increased up to twice normal concentrations during the first week. The 2 exceptions to this trend were patients with advanced cirrhosis, one of whom died of hepatic insufficiency. The increased alpha-globulin levels gradually declined to normal in over a month. The *beta globulin* levels varied considerably; the highest initial level occurred in a patient with pituitary adenoma who had an intracranial-paranasal fistula complicated by meningitis; and the lowest levels were in 2 patients with chronic alcoholism. During convalescence the beta globulin showed marked increases in 3 patients, one of whom was an elderly man with a history of congestive heart failure who suffered 3 relapses of pneumonia before a fatal outcome; and the other 2 were chronic alcoholics with pneumonia treated with antipneumococcal rabbit serum.

The *"fibrinogen fraction"* was considerably increased in all patients, and returned to normal during convalescence in only 2 patients. These 2 had pneumonia without any antecedent complications. The highest value of 1.3 g% was obtained just prior to death in the patient with meningitis and hepatic insufficiency. Two others, who showed progressive hyperfibrinogenemia, had chronic alcoholism, were severely ill with pneumonia, and showed jaundice. It should be noted that the fibrinogen values obtained by electrophoresis contained unknown amounts of gamma-1 globulin, so that the increases observed cannot be considered pure fibrinogen. Hence we have used the term "fibrinogen fraction."

Gamma globulin concentrations of greater than 0.9 g% were seen in the acute phase of the illness or in convalescence in 10 patients. Of these, 4 had chronic alcoholism; 3 had

[5] Zeldis, L. J., and Alling, E. L., *J. Exp. Med.*, 1945, **81**, 515.

[6] Rutstein, D. D., Thomson, K. J., Tolmack, D. M., Walker, W. H., and Floody, R. J., *J. Clin. Invest.*, 1945, **24**, 11.

hepatic cirrhosis; 2 had heart failure with hepatic congestion; and 1 had multiple myelomata. Thus the abnormal increments in the gamma globulin levels appeared to be related to underlying disease of the liver or bone marrow. In the electrophoretic fractionation of the plasma proteins in the myeloma blood, there was a protein having the mobility of gamma globulin with a concentration of 5.9 g% as determined by the area under the peak. When this patient's plasma was studied again 5 months later, there was no change in this peak. Only 2 patients had subnormal gamma globulin levels. Each of these gave a history of previous pneumonic infections several years before. Both were given 50 ml of immune human gamma globulin intramuscularly in addition to the routine treatment. Subsequent electrophoretic patterns of their blood showed increments in the gamma globulin fraction. Four out of 5 other patients similarly treated with intramuscular gamma globulin showed appreciable increases in the plasma concentrations of this fraction following the injection (Fig. 1). The amount of gamma globulin injected in these 7 patients represented about 7.0 g, and the maximum average increase in concentration was 0.45 g%, or about 65% of the average normal level. Absorption was delayed as there was no prompt initial rise. Since, however, 4 of the 7 so treated were known to have had chronic alcoholism and another had had decompensated heart disease, it is difficult to attribute the greater increments in this fraction to the material administered, although it is possible that the restoration of normal levels was significant in the only 2 patients with abnormally low initial gamma globulin levels. During convalescence there was a relative increase in the gamma globulin concentration in all the patients studied, but this was attributed in part to the concomitant decline in alpha-globulins.

Discussion. Pneumococcic infections, in common with other acute infectious diseases and severe bodily trauma,[3] initiate non-specific alterations in the protein metabolism. The present findings of hypoalbuminemia, increased alpha-globulins and fibrinogen are in accord with the findings of previous observers.[1,2] When evaluated chronologically, however, from the onset of the infection, it is apparent that the alpha-globulins and fibrinogen increase promptly and decline gradually in convalescence; whereas the hypoalbuminemia becomes progressively more severe, reaching the lowest levels in the second week of the infection and slowly is restored to normal over the following month. The intensity of the hypoalbuminemia, and in turn the hypoproteinemia, is considerably influenced by antecedent factors adversely affecting liver function, such as malnutrition, chronic alcoholism, cirrhosis and chronic passive congestion. Furthermore the severity of the electrophoretic hypoalbuminemia is greater than is recognized by chemical determinations. Dole[7] has shown that the Howe separation[8] is faulty, due largely to the solubility of much of the alpha-globulins in the concentration of sodium sulfate employed in the Howe technic. Hence the hypoalbuminemia tends to be obscured by the concomitant increase in alpha-globulins during acute febrile illnesses which are incompletely salted out by the clinical method of separation. The hypoalbuminemia in pneumonia may be the result of poor dietary intake of nitrogen, nitrogen catabolism, losses of protein in exudate and urine, and possibly impaired synthesis of protein. In experimental dogs, poor dietary protein intake and plasmaphoresis have been shown to diminish the albumin level and increase the alpha-globulins.[5]

Previous variations in the reported findings of gamma globulin concentrations in pneumonia[1,4] may have been due to the selection of patients and the relation to the onset of infection. Our findings indicate abnormally low levels early in the infection in occasional patients, a relative increase in concentration during convalescence, and an abnormally high level in those patients having impaired liver function prior to the onset of pneumococcic infection. The supplemental intramuscular

7 Dole, V. P., and Braun, E., *J. Clin. Invest.*, 1944, **23**, 708.

8 Howe, R. E., *J. Biol. Chem.*, 1921, **49**, 109.

injections of gamma globulin† may have been responsible for the increases in plasma levels which occurred in the 2 patients with low values.

Summary. Multiple electrophoretic determinations of the plasma proteins of 23 patients with pneumococcic pneumonia or meningitis have been made in relation to the duration of the infection and the importance of antecedent factors. Hypoproteinemia, due largely to significant progressive hypoalbuminemia, together with early increases in alpha-globulins and fibrinogen have been observed. Malnutrition, chronic alcoholism, cirrhosis and chronic passive congestion of the liver were found associated with more severe degrees of hypoalbuminemia. Diminished plasma albumin levels were seldom restored to normal within a month from the onset of the infection. The effects of intramuscular injections of immune human gamma globulin were investigated, with inconclusive results.

† Dr. W. Addison Clay assayed the protective power of gamma globulin in type 27 pneumococcic peritonitis in 40 mice and found a reduction of gross mortality from 95 to 55%. By serial dilutions the protective power was estimated to be of the order of one ten thousandth of that of immune rabbit serum.

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Effects of Albumin on Hypoalbuminemia in Pneumococcic Pneumonia.

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A previous report[1] confirmed the findings of others that patients with pneumococcic infection exhibit hypoalbuminemia within the first 2 weeks of the infection. Results obtained by electrophoresis of plasma proteins showed depression of albumin concentrations to as low as 1.4 to 1.8 g%, and that normal concentration was not often established within a month after the infection. Such marked hypoalbuminemia was considered to be the result of protein loss in exudate and urine in addition to protein break-down and malnutrition during the acute phase of the illness. Because of this hypoalbuminemia the effects of intravenous albumin therapy were investigated. This report presents the metabolic changes as well as some of the secondary effects of such therapy on cardio-respiratory functions.

Patients and procedures. During 1947 eight patients with severe pneumococcic lobar pneumonia were selected, and in addition to the routine chemotherapy of the infection 2 were controls, and 6 were treated with albumin. Observations on still another patient are included because of a fatal outcome following albumin therapy. The 6 albumin-treated patients and one control were placed on a special liquid diet† of known nitrogen content a day or two after the restoration of normal temperature following the febrile reaction. Twenty-four hour urinary collections were made to determine urinary nitrogen ex-

* Bertha Hochstetter Buswell Research Fellow in Medicine.

1 Bruce, R. A., Alling, E. L., and Barnett, A. D., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 398.

† The diet consisted of 1200 ml of powdered whole milk, eggs, yeast, and sugar in addition to orange juice. There was 17.7 g of nitrogen in this mixture. To satisfy some patients, the amount of diet had to be increased to 2400 ml volume.