

an intrinsic anti-tumor action of the drug.

1. Goldin, A., Burton, R. M., Humphreys, S. R., Vendetti, J. M., *Science*, 1957, v125, 156.
2. Belkin, M., Hardy, W. G., *ibid.*, 1957, v125, 233.
3. Belling, J., *Biol. Bull.*, 1926, v50, 160.

4. Kellicott, W. E., *Bull. Torr. Bot. Club*, 1904, v31, 529.
5. Levan, A., *Hereditas*, 1938, v24, 471.
6. Dustin, P., *Nature*, 1957, v159, 794.

Received March 4, 1958. P.S.E.B.M., 1958, v98.

Serum Protein and Glycoprotein Alteration in Swine with Experimental Arthritis.* (24008)

M. R. SHETLAR, CLARA L. SHETLAR, R. W. PAYNE, GEORGE M. NEHER AND
CHARLES B. SWENSON

*Departments of Biochemistry and Pharmacology, University of Oklahoma School of Medicine,
Oklahoma City and Department of Veterinary Science, Purdue University Agricultural
Experiment Station, Lafayette, Ind.*

A naturally occurring inflammatory arthritis of swine which frequently persists following systemic infection by *Erysipelothrix rhusiopathiae* has been described(1-4). A consistent finding in patients with active rheumatoid arthritis is an increased concentration of carbohydrate-rich globulins (serum glycoproteins)(5). Serum glycoprotein levels have been utilized as objective means of evaluating current inflammatory activity of patients with rheumatoid arthritis(6,7). The present investigation is concerned with the study of serum protein and glycoprotein changes in swine following the production of chronic arthritis by injections of *Erysipelothrix rhusiopathiae*.

Method. The incidence of arthritis following acute infection by this organism can be substantially increased by first vaccinating the swine with bacterins and later administering an intravenous challenging dose of *E. rhusiopathiae*. Under such circumstances approximately 90% of surviving animals develop the characteristic inflammatory arthritis(4). Swine of both sexes weighing approximately 200 lb were used. After intravenous injection of 10 ml of a 24 hour culture of *Erysipelothrix rhusiopathiae* in semi-solid medium, all animals developed acute swine erysipelas with high fever followed by death of approximately half of the animals. Initial blood samples were taken from 16 surviving

TABLE I. Effects of Swine Arthritis and Acute Erysipelas Infection on Protein-Bound Carbohydrates and Proteins of Swine.

No. of animals in group	Arthritic			Initial infection
	Normal 11	Inactive 4	Active 12	
Total serum glycoprotein (mg %)*	163 ± 6	162	229 ± 5 †	205 ± 4 †
Seromucoid fraction (mg %)*	12 ± .7	16	31 ± 1.6 †	29 ± 1.9 †
Total serum protein (g %)	7.17 ± .11	7.10	7.47 ± .12†	7.23 ± .09 †
(Total serum glycoprotein + total protein) × 100	2.27 ± .06	2.28	3.10 ± .06†	2.85 ± .06 †
[(Total serum glycoprotein-seromucoid) + total protein] × 100	2.10 ± .05	2.04	2.65 ± .04†	2.45 ± .03 †
Seromucoid + total glycoprotein	.075 ± .002	.099	.136 ± .006	.138 ± .007†

* Expressed as bound hexose (galactose-mannose).

† Significantly higher than normal value at 1% level of probability.

* This work was supported in part by research grants from Nat. Inst. of Arthritis and Metabolic Diseases, P.H.S.

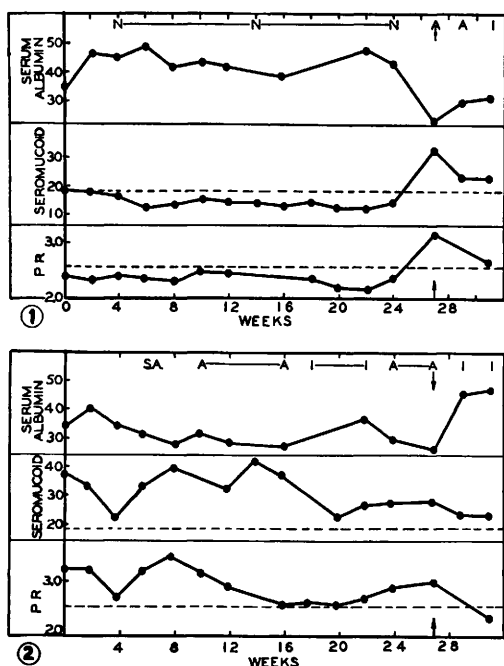


FIG. 1. Serial studies of an arthritic swine. Animal recovered from initial inflammatory stage and then developed acute arthritis at 27th wk, indicated by letter "A" at top of chart; N indicates lack of clinical signs of arthritis. Note increase of seromucoid and PR (serum glycoprotein: protein ratio) and decrease of serum albumin during the acute phase. Serum albumin is expressed as % of total serum protein, seromucoid, as mg of hexose/100 ml of serum, and PR, as g of bound hexose/100 g of total serum protein.

FIG. 2. Serial studies of an arthritic swine. Animal recovered from acute inflammation but rapidly developed arthritis (as indicated by A—A at top of chart). At 18 wk, remission occurred as indicated by I—I—I. Note decrease in seromucoid and PR (glycoprotein hexose: protein ratio) during this period, followed by exacerbation of the disease on 24th wk. On 27th wk this animal was treated with cortisone with clinical improvement. Serum albumin is expressed as % of total serum protein, seromucoid, as mg hexose/100 ml of serum, and PR, as g of bound hexose/100 g of total serum protein.

animals 17 days post exposure, and comparisons were made to sera of 10 unexposed control swine. The body temperature of exposed animals returned to normal 3-8 days before the sampling. Subsequent samples were taken every 2 weeks for 31 weeks. Total serum glycoprotein hexose was determined by the tryptophan method of Shetlar, Foster, and Everett(8), and serum protein by the biuret method. Seromucoid was isolated by the method of Weimer and Moshin(9) and quan-

titated as hexose by the tryptophan method. Paper electrophoretic studies were made as previously described(7) using 3 MM filter paper and the Spinco hanging strip electrophoresis apparatus.

Results. Serum glycoprotein and protein concentrations in normal and arthritic swine in the active phase are summarized in Table I. The effects of the initial acute erysipelas infection on serum glycoprotein and protein as found on the first bleeding are also shown, as are findings in those arthritic animals whose disease had become clinically inactive. Serum glycoprotein and seromucoid levels were significantly elevated in all animals following erysipelas infection. Similar elevations were also noted in those animals which developed chronic arthritis after the initial phase of the disease. Serum glycoproteins remained significantly elevated even after that portion due to seromucoid was deducted from the total glycoprotein bound hexose. However, it is noteworthy that the ratio of seromucoid to total serum glycoprotein was increased significantly by acute erysipelas, as well as by the ensuing chronic arthritis. Swine with inactive arthritis exhibited serum glycoprotein and seromucoid concentrations within the range normal for swine.

Results of serial studies of 2 swine are graphically shown in Fig. 1 and 2. Fig. 1 summarizes data of animals which appeared to recover from the initial infection and then developed acute arthritis during the 27th week of study. When the clinical condition improved, a decrease in serum glycoproteins and an increase in serum albumin was noted. Fig. 2 illustrates data concerning an animal which recovered from early acute inflammation, then rapidly developed arthritic symptoms. Cortisone treatment (300 mg/day) was started on the 27th week producing clinical benefit, paralleled by a decrease in serum glycoproteins and an increase in serum albumin as described above.

Definite α_1 -globulin peaks were not noted in any of the swine paper electrophoretic patterns. This is in agreement with electrophoretic patterns of pig serum obtained by Deutsch(10) using a Tiselius technic.

TABLE II. Summary of Changes in Glycoprotein Hexose and in Serum Protein following Administration of Cortisone to Arthritic Swine.

	Before cortisone	After cortisone
	Bound hexose as mg/100 ml	
Glycoprotein		
Total	202	174
Seromucoid	21	15
α -globulin	95	89
β - "	48	39
γ - "	49	31
Albumin	11	14
	Protein as g/100 ml	
Protein		
Total	7.48	8.02
α -globulin	1.26	1.30
β - "	1.38	1.21
γ - "	2.58	1.58
Albumin	2.26	3.94

This absence of α_1 -globulin in the arthritic swine is particularly interesting as this fraction exhibits striking changes in the human patient with active rheumatoid arthritis. The γ -globulin was found to be elevated (compared to the human) even in patterns of control swine. After cortisone treatment the γ -globulin fraction was reduced below that of the control animal. Glycoprotein electrophoretic studies of the sera of the various groups indicated that elevations of bound carbohydrate attached to the α -, β - and γ -globulin fractions occur in the acutely arthritic animals. A decrease of these components occurs after cortisone administration (Table II), being particularly evident in the γ -globulin and seromucoid fractions. No change occurs in the α -globulin fraction if the assumption is made that all of the seromucoid is found in the α -globulin after electrophoresis.

Summary. A serial study was made of pro-

tein and glycoprotein changes of swine during arthritis which follows recovery from *Erysipelothrix* infections. As in sera from humans with rheumatoid arthritis, elevated serum glycoprotein, seromucoid and serum globulins and lowered serum albumin concentrations were noted in swine sera during active phases of the disease. No serum α_1 -globulin was identified in swine sera. During remissions of experimental arthritis, serum glycoprotein levels decreased and the serum albumin increased. Cortisone treatment of animals with active arthritis produced clinical improvement accompanied by striking increases of albumin protein with decreases of seromucoid, serum γ -globulin protein and γ -globulin bound hexose concentrations.

1. Collins, D. H., Goldie, W., *J. Path. and Bact.*, 1940, v50, 323.
2. Doyle, L. P., *Vet. Med.*, 1951, v46, 257.
3. Sikes, D., Neher, G. M., Doyle, L. P., *Am. J. Vet. Res.*, 1955, v16, 349.
4. ———, *ibid.*, 1955, v16, 367.
5. Shetlar, M. R., Payne, R. M., Bullock, J. A., Patrick, D. R., Hellbaum, A. A., Ishmael, W. K., *J. Clin. Invest.*, 1953, v32, 1208.
6. Payne, R. W., Shetlar, M. R., Bullock, J. A., Patrick, D. R., Hellbaum, A. A., Ishmael, W. K., *Ann. Int. Med.*, 1954, v41, 775.
7. Stidworthy, G., Payne, R. W., Shetlar, C. L., Shetlar, M. R., *J. Clin. Invest.*, 1957, v36, 309.
8. Shetlar, M. R., Foster, J. V., Everett, M. R., *Proc. Soc. Exp. Biol. and Med.*, 1948, v67, 125.
9. Weimer, H. B., Moshin, J. R., *Ann. Rev. Tuberc.*, 1953, v68, 594.
10. Deutsch, H. F., Goodloe, M. B., *J. Biol. Chem.*, 1945, v161, 1.

Received March 10, 1958. P.S.E.B.M., 1958, v98.