arthritis(3). Despite the apparent lack of immunity to PPLO growing in tumor tissue, rats bearing PPLO-infected or PPLO-free tumors were relatively resistant to PPLO polyarthritis. Tumor transplants to rats with established chronic PPLO polyarthritis, did not become infected with PPLO(3). The present study suggests that circulating antibodies prevented spread of PPLO to the recently implanted tumor.

The role of the 2 morphological types of PPLO in polyarthritis is uncertain. No attempt was made to establish their interconversion and both types were pathogenic although the diffuse form produced more lesions. Following intravenous injection, pathogenic Mycoplasma rapidly disappeared from tissue but apparently lodged in synovial structures, establishing suppurative arthritis (6). Because of the unreliability of cultural and turbidometric methods for estimating numbers of PPLO the role of aggregation of these minute microrganisms upon their tissue distribution after intravenous injection, and hence upon the incidence of joint disease, remains unknown.

Summary. The ability of Mycoplasma (PPLO) to produce polyarthritis in rats has been modified *in vitro* and *in vivo*. Pathogenic PPLO recovered from Murphy-Sturm lymphosarcomas became non-virulent upon repeated subculture in commercial media, but the pathogenic properties were restored and maintained by addition of cell- and bacteria-free extract of the tumor tissue. Growth of Mycoplasma in tumor tissue *in vivo* also restored the pathogenic property. Diffuse and clonal forms of the pathogenic PPLO were isolated. Twice as much interphalangeal polyarthritis followed injection of the diffuse as the clonal form. Prior PPLO arthritis resulted in immunity. Passive transfer of the immunity was accomplished with serum but not with splenic cells.

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Electrophoretic Studies of Red Cell Hemolysates Supplemented with Phosphorylated Carbohydrate Intermediates.* (27953)

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During the storage of blood at 4°C in acid citrate dextrose (ACD) preservative, concentrations of the organic phosphates, particularly 2,3-diphosphoglycerate and adenosine triphosphate, of the red cell decrease and inorganic phosphate increases(1). Under these same conditions of storage, one of the electrophoretic components of the red cell hemolysate, designated as B, gradually disappears(2). It is conceivable that this component may be composed of a complex made up of phosphorylated carbohydrate intermediates and hemolysate proteins. The present experiments were undertaken to determine if the distribution of the components of the electrophoretic patterns of red cell hemolysates is changed after adding phosphoric acid esters.

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Materials and methods. The red cells of ACD blood, stored at 4°C for about a month, were washed 3 times with cold 0.9% NaCl and then hemolyzed with distilled water. Stroma was removed by high speed

centrifugation and the clarified hemolysate was dialyzed against 0.1 M NaCl for 18 hours. It was observed that this dialysis reduced both the organic phosphate and the Component B concentrations. The following materials were added to 4 ml portions of hemolysates diluted to contain 300 mg hemoglobin (Hb); 2,3-diphosphoglycerate (DPG), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), 3-phosphoglyceric acid (3PGA), fructose-1,6-diphosphate (FDP), glucose-6-phosphate (G6P) and inorganic phosphate (P_i) as a phosphate buffer at pH 7.0. Concentrations of these compounds are expressed as micromoles per g Hb. After adding the desired amount of material to each tube, the volume was brought to 10 ml with water and the mixture stored overnight in the refrigerator. After these hemolysates were dialyzed against 3 changes of cacodylate buffer (ionic strength 0.033, pH 6.5) during a 24-hour period, Hb concentration was adjusted to 2% by adding buffer. An aliquot was used for measuring P_i and total phosphate(3). Electrophoresis proceeded for 150 minutes in a Tiselius apparatus using an 11 ml cell. A photograph was taken by Longsworth's scanning technic(4) immediately after the current was shut off. The current was then reversed for 35 minutes and the material was allowed to diffuse for 15 minutes at which time a second photograph was obtained. Tracings of the ascending patterns, magnified 3 times, were made. The areas of Components B, x and f were delineated and measured by planimetry from the tracings of the first photograph. The total area of the pattern was determined from the second photograph; Component A was estimated by difference between this area and the sum of the areas of the 3 components mentioned.

Results. The greatest changes in the electrophoretic patterns of red cell hemolysates are observed after addition of DPG, ATP and FDP. The patterns and percentage distribution of the 4 components (B, x, A and f) are shown in Fig. 1, 2 and 3.

Addition of a small amount of DPG (5 μmoles/g Hb) causes a 3-fold increase in Component B concentration and a decrease

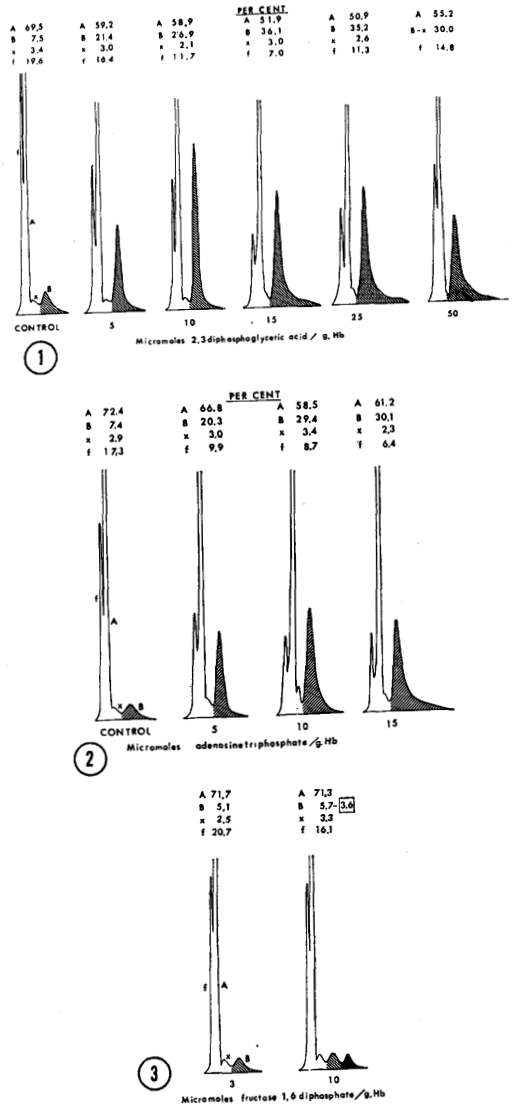

FIG. 1. Electrophoretic patterns of a red cell hemolysate which had been incubated with varying amounts of DPG and percentage distribution of components. Shaded area represents Component B.

FIG. 2. Effect of adding ATP to hemolysate on electrophoretic pattern.

FIG. 3. Effect of adding FDP to hemolysate on electrophoretic pattern.

in both Components A and f (Fig. 1). After adding 10 and 15 μmoles, further increases in Component B are seen. The distribution of the 4 components in the 15 and 25 μmole groups approximate the values obtained for a red cell hemolysate of freshly drawn blood which are 34.5, 54.6, 7.2 and 3.3 per cent of

TABLE I. Concentration of Organic Phosphate (μmoles/g Hb) After Incubation with DPG and ATP.

DPG added, μmoles	Organic phosphate, μmoles	DPG,* μmoles	ATP added, μmoles	Organic phosphate, μmoles	ATP,* μmoles
0	7.3	—	0	9.7	—
5	14.4	3.6	5	21.8	4.0
10	21.2	7.0	10	35.1	8.5
15	31.7	12.2	15	44.4	11.6
25	34.2	13.5			
50	40.1	17.8			

* Calculated from increase over control value: divide by 2 or 3 for DPG or ATP.

the total pattern area for Components B, A, f and x, respectively. In the presence of relatively large amounts of DPG (50 μmoles), Component x is no longer present. In Table I, it is observed that the organic phosphate concentration of the dialyzed hemolysates increases rapidly after adding small amounts of DPG and more slowly with larger quantities. It is obvious that the changes in distribution of the various components do not parallel the increased concentrations of DPG.

After supplementing the hemolysate with 5, 10 and 15 μmoles ATP (Fig. 2), the increases in Component B concentration are somewhat smaller but approximate the values obtained after adding equimolar concentrations of DPG. It was observed that Component x was no longer present after adding 25 and 50 μmoles of ATP. The organic phosphate concentrations of the ATP group are definitely greater than those of the equimolar DPG samples (Table I). Analyses were not performed to determine the extent to which ATP might be transformed to secondary products.

A small amount (3 μmoles) of FDP has no effect on the pattern but larger quantities (10 μmoles) are responsbile for the appearance of an added component on the trailing edge of Component B which represents 3.7% of the total pattern area (Fig. 3). FDP is the only compound which yielded this type of pattern.

Additional compounds. The percentage distributions of the electrophoretic components after supplementing hemolysates with ADP, 3PGA, AMP, G6P, and P_i are shown in Table II. Small but appreciable increases in concentration of Component B are noted after adding ADP and 3PGA. A small but definite decrease in Component B is seen in the G6P and P_i groups while no change is discernible in the AMP sample.

All experiments were run on at least 2 different hemolysates.

Discussion. It has been observed in this laboratory that the concentration of Component B decreases and Components A and f increase during storage of ACD blood in the cold. Conversely, in the present experiments, concentrations of Component B increase and Components A and f decrease after a hemolysate is incubated with DPG and ATP, the 2 phosphorylated compounds present in largest amounts in the red cell. These observations could be explained if it is assumed that phosphoric acid esters interact with protein to form a complex which is readily dissociated or regenerated. Under such conditions the charge and mobility of the protein would be affected and this would account for the changes in con-

TABLE II. Effect of Adding Phosphorylated Carbohydrate Intermediates and Inorganic Phosphate to Hemolysates on Percentage Distribution of Electrophoretic Components.

Compound	μmoles added	Components A	B	x	f	Organic phosphate, μmoles/g Hb
		%				
Control	—	69.5	7.5	3.4	19.6	9.6
ADP	5	66.8	12.7	2.9	17.6	12.5
ADP	10	63.8	16.5	3.0	16.7	21.9
3PGA	5	69.8	10.8	2.2	17.2	7.6
3PGA	10	69.2	10.4	2.3	18.1	11.2
AMP	10	68.1	8.6	3.6	19.7	9.0
G6P	5	72.6	3.9	2.4	21.1	9.5
G6P	10	72.7	5.9	2.3	19.1	8.9
P_i	10	72.7	5.0	2.5	19.8	8.6

centration of Components B, A and f.

Summary. Hemolysates of aged red cells were analyzed by electrophoresis before and after incubation with a number of phosphorylated metabolic intermediates. There was a marked increase in concentrations of one of the components (B) and a reduction in the amount of 2 components (A and f) in presence of added 2,3 diphosphoglycerate and adenosine triphosphate. Small increases in Component B concentration were observed in the adenosine diphosphate and 3-phosphoglyceric acid groups. A new electrophoretic component appeared after adding fructose 1,6-diphosphate.

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Normal Response to Erythropoietin or Hypoxia in Rats Made Anemic with Turpentine Abscess.* (27954)

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It is apparent that the anemia which develops in the presence of inflammation has a complex etiology. Disturbances in iron metabolism result in a markedly lowered plasma iron concentration and shunting of iron to storage pools(1,2,3,4). Wintrobe(5) has shown that the anemia of inflammation can be abolished by administration of cobalt, the action of which is assumed to be through stimulation of erythropoietin production(6). Yet in a preliminary study, Erslev(7) obtained evidence suggesting that both the production and effectiveness of erythropoietin were defective in the presence of inflammation. The present study was designed to clarify the role of erythropoietin in the etiology of the anemia which follows the production of a sterile turpentine abscess by testing the ability to respond to both exogenous erythropoietin and endogenous erythropoietin (hypoxia).

Materials and methods. Specific pathogen free, adult, female rats of the Sprague-Dawley strain weighing approximately 200 g and fed *ad libitum* on Diet 1† were used throughout the study except that rats of the Long-Evans strain were used to make the erythropoietin dose-response curve(8). Turpentine abscesses were produced by injection of N.F. oil of turpentine into the gluteal muscle (0.125 ml on days 1 and 2 and 0.250 ml on day 7). On day 15, the rats were weighed, and total circulating red cell volume and hemoglobin were determined by the Fe^{59} labeled red cell dilution method(9). Leucocytes and reticulocytes were counted. Bone marrow smears were made from femoral marrow diluted with rat serum. Weight of the spleen was recorded. Determination of plasma iron and total iron binding capacity of the plasma was done on plasma pooled from rats of the same group(10).

Erythropoietin was obtained by the collodion adsorption method(11) from the urine of a patient who had complete red cell aplasia. It had been assayed by a variety of methods(8). One-half or 2.5 C.S.‡ units

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† The diet, obtained from Simonsen Laboratories, Gilroy, Calif., consisted of 59.0% wheat, 11.7% skim milk, 11.2% casein, 11.2% rice bran, 3.3% vegetable oil, 1.3% $CaCO_3$, 0.7% NaCl, and vitamin and mineral mixtures to 100%.

‡ Comparative Standard(12).