TABLE I. Oxygen Consumption of Post-Mortem Human Heart Muscle.

Clinical diagnosis	Post-mortem interval, hr	Q_{O_2} Prep. A	Isometric developed tension, mg Prep. B
Hydrocephalus (still-born infant)	1.8	23	90
Rheumatic heart disease, mitral stenosis	2.0	17	1020
Generalized arteriosclerosis, involvement of coronary arteries	3.5	12	30
Arteriosclerotic heart disease	4.1	17	190
Generalized arteriosclerosis, G-I bleeding	5.0	27	150
Carcinoma of esophagus	5.5	21	55
Malignant carcinoid	8.4	31	475
Mean		21.0 ± 2.6	287 ± 137

of human cardiac physiology and pathophysiology.

Summary. Oxygen consumption measurements were made on trabeculae carneae from 7 contractile human hearts, obtained 1.8 to 8.4 hours after death. Mean QO_2 was 21.0 $\pm$ 2.5, which compares favorably with mean *in vivo* oxygen utilization values reported for the normal human heart. There was no correlation between magnitude of the oxygen uptake and isometric tension developed by a sister preparation from the same heart, nor with the post-mortem interval.

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Isolation of Partially Purified Properdin from Bovine Serum by Cold Ethanol Fractionation.* (24311)

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Properdin, a normal serum constituent which in conjunction with complement or complement-like substances and Mg++ constitutes the properdin system, was first reported to be a natural humoral defense mechanism by Pillemer and co-workers(1). A procedure for isolation and concentration of this euglobulin substance was described. The protocol involves the adsorption of properdin to zymosan and subsequent elution under suitable conditions(2). The use of the zymosan adsorption technic generally yields final properdin solutions of variable activity since zymosan preparations vary in activity. Pillemer *et al.*(1) reported that properdin is found in serum Fraction III using the separation procedure described by Deutsch *et al.*(3). Pennell *et al.*(4) used method 6 of Cohn, *et al.*(5) and reported that 90-95% of total properdin activity of plasma was recovered in a subfraction of Fraction I, and the remaining activity appeared in Fraction III. Linder(6) has shown that the majority of properdin activity of human serum resides in the gamma globulin eluate when the serum constituents are separated electrophoretically. Experiments designed to determine the value of properdin as a therapeutic agent following exposure of animals to whole body x-irradiation made necessary the preparation of properdin solutions of high activity per unit volume in order that

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adequate concentrations of material could be administered intravenously or intraperitoneally without causing physiological stress in mice and rats. A Cohn ethanol fractionation (modified Method 6) procedure was employed in order to obtain partially purified properdin solutions without using the variable and costly zymosan. The results of a direct ethanol fractionation procedure are reported herein.

Materials and Methods. Bovine serum. The whole blood of healthy adult cows was collected in sodium dichromate-sulfuric acid cleaned glass receptacles and allowed to stand at room temperature for 2 hours. The clots were then rimmed, and the blood stored overnight at 2°C. The serum was separated by centrifugation at high speed in a refrigerated centrifuge. The clear serum was used immediately in the fractionation procedure. *Ethanol-buffer mixture.* For each liter of serum the following solution was prepared: 601 ml of 53.3% ethyl alcohol at −5°C; 0.88 ml of 10 M acetic acid at 25°C; 0.44 ml of 4 M sodium acetate at 25°C; 2.30 ml of 95% ethyl alcohol at −5°C. The above ingredients are measured out at the temperature specified and mixed together thoroughly. The entire mixture was allowed to equilibrate at −5°C before addition to the serum. *pH 7.4 Barbital buffer* was prepared according to the directions in Kabat and Mayer(7). *Constant temperature bath.* A double jacketed stainless steel container was employed. The smaller inner jacket contained a solution of sodium chloride with a freezing point depression of −5°C to −6°C. The larger outer jacket contained the freezing calcium chloride-ice mixture. *Procedure.* The glass container holding the clear bovine serum was placed into NaCl solution. The serum was stirred slowly with a mechanical agitator to hasten equilibrium conditions and prevent ice crystal formation. When temperature of the serum was 0°C, the pre-cooled (−5°C) ethanol buffer mixture was added. Rate of addition through a capillary jet was 5-6 ml per minute. Overall addition time per liter of serum was about 2 hours. The entire mixture was adequately stirred during the addition of the ethanol buffer mixture in order to prevent high ethanol concentrations near the jet tip which could cause irreversible protein changes. The final concentration of ethanol was 20% V/V. After addition of the ethanol buffer solution the mixture was continually stirred for another 60 minutes. Care was exercised to keep the serum-ethanol mixture at a temperature of −5 ± 0.5°C. The precipitate was centrifuged out at high speed in a refrigerated centrifuge (0°C). The supernate was discarded, and the centrifuge tubes were inverted 10 minutes in the refrigerator to drain the excess fluid. The precipitate was redissolved in pH 7.4 barbital buffer, (250 ml buffer/liter starting material). The redissolved protein fraction was transferred to a filtering flask, and negative pressure was applied to remove the residual alcohol. This operation was carried out at room temperature. The protein solution was cooled to 0°C for 24 hours during which time a precipitate appeared. The mixture was centrifuged and the residue collected. The supernate was discarded. The residue was treated with pH 7.4 barbital buffer (3 ml buffer per ml of residue). Suspension of the residue was obtained by gentle agitation at room temperature. The final product was stable turbid suspension and was stored at 0°C. The titers of the properdin mixtures at various steps in the isolation are shown in Table I.

TABLE I. Properdin Activity of Various Fractions Obtained during Isolation Procedure.

Material	Amt, ml	Properdin,* units/ml	mg N per ml
Bovine serum	1000	15	18.7
↓			
Redissolved ethanol precipitation fraction	250	100–200	27.5
↓			
Supernate from cold separation centrifugation	235	2–4	12.7
Residue from cold separation centrifugation	15	400	37.6
↓			
Final suspension	60	100	9.4

* Zymosan assay procedure of Pillemer *et al.* (1956).

Results. It is seen that considerable increase in activity on a volume basis was

achieved, *i.e.*, from 15 to 100 units per ml. However, the isolating procedure is carried out at a net loss, *i.e.*, from 15000 units to 6000 units or a net recovery of about 40%. The value of the procedure derives from the increased concentration per unit volume.

Anticomplementary titers of the products were run in conjunction with the properdin assays and in each case the product was shown to be non-anticomplementary. The final product had the paper electrophoretic characteristics of gamma globulin.

The purification step achieved by inadvertent discovery of the insolubility of the properdin-containing fraction at 0°C was apparently made possible by the prior concentration steps. The possible relation of "cryogenic" proteins(8,9,10) to the partially purified properdin. which acts similarly to described cryogenic globulin, remains to be determined.

Summary. A procedure for isolation of properdin from bovine serum using cold ethanol fractionation technics is reported. Results indicate that high yields of properdin per unit volume are obtainable without use of zymosan.

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Sensitized Sheep Cell Hemagglutination Reaction in Rats with Experimental Infection of Bone and Joint. (24312)

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A reproducible infection of bone and joint of rats has been produced in high incidence with a strain of *Streptobacillus moniliformis* isolated from a rat with middle-ear infection (1). Although this microorganism is considered of low virulence or a commensal for rats(2,3), this strain isolated in our laboratory has shown a specific affinity for joints, bone, and periarticular tissues after intravenous injection. To date, 109 out of 116 animals injected intravenously with this microorganism have developed gross and/or microscopic lesions. Of those animals showing acute changes, the infecting microorganism has been recovered from 19 of 21 wrists or ankles cultured during the acute, 5-11 day stage. Blood cultures made during this same 5-11 day stage have been positive in only 1 of 15 animals. The involvement of the region of the joint in the inflammatory process has directed immunologic studies of this infection into investigation of possible similarities to serologic reactions seen in human rheumatoid arthritis. Observations that the serum of patients with rheumatoid arthritis agglutinated sensitized bacteria, red cells, and other particles(4,5,6), have led to development of the sensitized sheep cell agglutination (SSCA) reaction as a phenomenon occurring most frequently in rheumatoid arthritis(7,8,9,10). This agglutination reaction has been found positive less frequently in some of the so-