crease in hematocrit to 51% as compared with a normal value of 42-45%. Animals receiving no propylene glycol showed no increase in hematocrit above the normal value. p-Chloroacetanilide, 100 mg/kg, dissolved in propylene glycol, 4 ml/kg, produced methemoglobinemia in both species; to a greater extent in the rat than in the guinea pig: 8.1% and 4.2% of the total blood pigment, respectively. Propylene glycol alone, 4 ml/kg, produced no increase in methemoglobin above normal range: 1.5% for rats, and 2.2% for guinea pigs. p-Chloroacetanilide, 100 mg/kg, in aqueous suspension increased the methemoglobinemia in both species; the increase more pronounced in the rat than in the guinea pig: 40.8% and 6.9% of the total blood pigment, respectively. These results indicate a decrease in production of the methemoglobin-producing metabolite of p-chloroacetanilide in the presence of propylene glycol. It is suggested that results attributed to a compound administered in propylene glycol solution should be carefully interpreted since the propylene glycol may make a marked contribution to the physiological activity of the mixture.

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Synovial Fluid. I. Comparison of Sodium and Potassium Concentrations in Normal and Diseased Joint Fluid.* (20986)

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(Introduced by Ward Pigman.)

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The finding of excess synovial fluid in various disease states has prompted us to investigate the sodium and potassium concentrations of fluids obtained from arthritic joints and compare them with those obtained from the normal. Previous investigations on synovial fluid have utilized material obtained from cattle(1,2), horses(3), dogs(4), edema effusion(5), and human autopsy material (2,6,7).

Material and methods. The synovial fluid samples were aspirated from the knee joints of 10 normal living subjects, 10 autopsy cases, 25 patients with effusions in untreated acute rheumatoid arthritis, and 5 individuals with a diagnosis of osteoarthritis with effusion. After repeated failure to obtain fluid from living subjects, it was found that by allowing the subjects to remain inactive for 8-12 hours prior to the procedure, volumes of fluid up to one cc could be obtained from the knee joint with only moderate difficulty. A 20-gauge needle was introduced under the medial border of the patella directly into the joint cavity. Prior infiltration of the skin with 1% procaine made the procedure relatively painless. The

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TABLE I. Na and K Concentrations in mEq/L in Normal and Diseased Joints.

Cases	No.	Na	K
Normal living	10	136.1±1.63	4.0±.25
" autopsy	10	133.7±3.36	4.8±.13
Rheumatoid arthritis	25	143.6± .98*	4.6±.09
Osteoarthritis	5	136.9±1.95	4.1±.10

Synovial fluid/serum ratios for Na and K

	Na	K
Normal living	.93±.011	1.08±.09
Rheumatoid arthritis	.99±.010*	1.03±.04
Osteoarthritis	.94±.014	.95±.12

* Significantly different from normal.

fluid obtained from individuals with rheumatoid arthritis was first allowed to clot and then centrifuged before analysis. Sodium and potassium concentrations were determined using the Beckman DU flame photometer. In order to achieve the necessary uniform viscosities and aspiration rates for all samples, Wydase‡ was added to each solution at the rate of 50 TR units per cc of synovial fluid.

Results. Comparative results are shown in Table I. The average sodium concentration in normal synovial fluid was 136.1 mEq/1, with a fluid/serum ratio of 0.93 (0.89-0.98). The ratios for potassium ranged from 0.70-1.70, with an average concentration in the joint fluid of 4.0 mEq/1. Average sodium and potassium values from autopsy fluids were 133.7 and 4.9, respectively.

Synovial fluids obtained from the patients with rheumatoid arthritis revealed average sodium and potassium values of 143.6 and 4.6 mEq/1, with fluid/serum ratios of 0.99 (0.95-1.03) and 1.03 (0.92-1.19). Fluid obtained from osteoarthritic joints had average concentrations of 136.9 for sodium and 4.1 for potassium and fluid serum ratios of 0.94 sodium and 0.95 potassium, respectively. Statistical analysis of the data revealed a difference of 3.95 standard errors between the mean sodium concentration in normal synovial fluid and that of rheumatoid arthritics. In view of the wide range in fluid/serum ratio for potassium in normal fluids, little significance could be attributed to this phase of our investigation.

‡ Trade Name—Hyaluronidase—Wyeth Laboratories, Philadelphia, Pa.

Discussion. Ropes and Bauer have determined the sodium and potassium distribution ratios between serum and synovial fluid in cattle(2). The fluid/serum ratio for sodium was 0.93 and that for potassium was 0.75. Their findings appear to correlate closely with the sodium values obtained in this study of normal and autopsy material.

The increase in the sodium concentration in the joint fluid of patients with rheumatoid arthritis as compared to that found in normal fluids appears to be statistically significant. This increase in synovial fluid sodium concentration can be considered to be of more significance since the serum values were essentially equal in normal subjects and those with rheumatoid arthritis. This is doubly substantiated by a previous study of serum sodium and potassium values in rheumatoid arthritis (8), in which they were essentially equal to the reported normals(9).

There appear to be at least 3 possible factors affecting the alteration in the electrolyte concentrations of synovial fluid in diseased states; *i.e.*, alteration of protein content, qualitative and quantitative alteration of the polysaccharide component of the fluid, and changes in permeability of the synovial membrane. It has been shown electrophoretically that the total protein content and especially that of the globulin fraction is increased in the joint fluid in rheumatoid arthritis(10). More recently it has also been shown that there are extensive qualitative alterations in the protein components as evidenced by the changes in their mobilities seen in the electrophoretic pattern(11).

Conclusions. 1. Data have been presented on the sodium and potassium concentrations of normal human synovial fluid. From the values obtained it is apparent that the normal values for sodium previously reported on material obtained from cattle joints compares favorably with those found in the living human subject and in postmortem fluids. The potassium values were quite variable, using the present technics. In the synovial fluid obtained from rheumatoid arthritics, there was an increase in sodium concentration with a corresponding increase in the synovial fluid/serum ratio for sodium. 2. Technics are de-

scribed for the collection and analysis of synovial fluid.

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Effects of Storage on Eastern Equine Encephalitis Virus. (20987)

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Because of a lack of adequate information on the preservation of virus in field and laboratory specimens, an investigation was undertaken to explore, in part, the effects of various storage conditions upon eastern equine encephalitis virus (EEE). Some of the studies were designed to determine effects of different insect killing methods and storage temperatures upon EEE virus in infected, intact mosquitoes. Others were intended to measure the effects of carbon dioxide gas in dry ice storage upon virus in intact mosquitoes and mouse brains as well as mosquito and mouse brain suspensions. Stabilizing properties of several types of diluents on virus-containing mosquito and mouse brain suspensions were also investigated.

Materials and methods. The mosquitoes (*Aedes aegypti*) were infected with a recently isolated strain of EEE by feeding upon infected chicks and used after 12 days extrinsic incubation at 80°F. Twenty-five specimens from the infected pool inoculated into mice were all found to contain virus, which indicated uniform infection of the pool. Pools of 5 mosquitoes were used for each unit of the intact mosquito studies. After storage, each pool of 5 specimens was ground in 1.0 ml of 0.1% bovine albumin-buffered saline diluent (pH 7.2) and centrifuged 10 minutes at 2500 RPM. The supernatant was decanted into vials in an ice bath, 2.0 mg of streptomycin and 1000 units of penicillin per ml were added and serial 10-fold dilutions made in cold 0.1% bovine albumin-saline. These dilutions were inoculated immediately via the intracerebral route into 3-week-old CFW mice to determine the virus titers, with the suspension of 5 mosquitoes in 1.0 ml considered as concentrated material or 10^0 dilution. Virus titers in all tables are expressed as the inverse log of the dilution killing half the animals inoculated. When mosquito suspensions rather than intact mosquitoes were stored, they likewise were prepared in the ratio of 5 mosquitoes per ml of diluent. Infected mouse brains were stored as 10% suspensions and as intact brain tissue.

Experimental. Effects of various insect killing methods upon EEE virus in infected mosquitoes. To determine whether the most common methods of killing field-caught mosquitoes affected EEE virus they might contain, infected mosquitoes were killed in the following ways: 1) Exposed to ether fumes for 2 minutes. 2) Exposed to chloroform fumes for 2 minutes. 3) Exposed to chloroform fumes for 2 minutes, plus wetting of one specimen of each pool of 5 with chloroform. 4) Quick-frozen on dry ice in contact with