calcium, phosphate, uric acid and creatinine). Furthermore, body weight decreases on succeeding days of the fast and possibly the intercorrelations also change. It does not seem justifiable, therefore, to express excretion rates of fasting rats on a body weight basis until much more information is available.

Conclusion. Young male rats that differed in body weight by only about 20% had significant ($p \leq .05$) metabolic differences during a 3-day fast. These included calcium, phosphate, urea, creatinine, the Na/K, Mg Ca, Ca/PO_4 and uric acid/creatinine ratios. There was an almost 10-fold difference in their respective growth rates, however, which undoubtedly contributed to the differences. These group differences tended to disappear in almost all variables as the fast progressed. The various ratios proved to be quite sensitive to the group differences.

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A Hemolytic Phenomenon in Ewes Requiring *Leptospira pomona* Antigen and Antibody.* (26415)

RAYMOND L. MORTER† (Introduced by E. V. Morse)

Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames

The properties of *Leptospira pomona* responsible for the hemoglobinemia and hemoglobinuria observed in field and experimental infections of cattle and sheep have not been defined(1,2,3,). *In vitro* studies have revealed the presence of hemolytic factors in the culture supernatant fluid(4,5,6). Little hemolytic activity was observed in preparations of washed disrupted cells(4,6). The hemolytic factor was thermolabile (inactivated at 56°C in 10 minutes) inhibited by specific antiserum, independent of complement and most active against erythrocytes of ruminants. Intravenous administration of the material produced marked and fatal hemolysis and hemoglobinuria in nonimmune lambs(7,8) Specific *L. pomona* antibodies inhibited hemolysis and protected lambs(7). The similarity of the effect of the hemolytic factor in lambs to the syndrome observed following active *L. pomona* infections has been reported(8).

During experimental infections in cattle and sheep, *L. pomona* has been isolated from the blood stream after the appearance of detectable specific antibodies. Ferguson *et. al.* (9) suggested that a hemolytic toxin was released from the leptospirae by the effect of a lytic antibody. Morse *et al.*(3) suggested a similar phenomenon as the cause of hemolytic anemia in experimental *L. pomona* infections of sheep.

Hemolysis and hemoglobinuria in cattle and sheep have occurred after the appearance of specific antibodies. Inhibition by specific leptospiral antibodies of the hemolysin from culture supernatant fluids suggests that another factor is responsible for hemolysis in actual infections. This preliminary investigation was undertaken to delineate the relation

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† Present address: Dept. of Microbiology, Pathology, and Public Health, School of Vet. Sci. and Med., Purdue Univ., Lafayette, Ind.

between *L. pomona* cell suspensions and hemolysis in immune sheep.

Materials and methods. Preparation of cell suspensions. *L. pomona* (strain LW) (10) was cultivated in Stuarts (Difco) medium containing 10% gamma-globulin-free sterile rabbit serum. Cultures were incubated for 10 to 12 days at 28°C, refrigerated at 5°C for 5 days and centrifuged at approximately 3500 g. The sedimented leptospirae were washed 3 times with approximately 15 cc sterile 0.85% saline, resuspended to approximately 1% of the original culture volume in sterile saline and stored at −30°C which rendered the leptospirae non-viable. The cells were disrupted by grinding in a motor driven, 40 ml Ten Broeck tissue grinder placed in an ice bath. After grinding for 15 minutes, no intact cells were observed in the resulting suspension by darkfield examination (525x).

Experimental animals were five apparently normal, mature, crossbred ewes. No *L. pomona antibodies* were detected in the sera of the ewes by modified agglutination-lysis test procedures(11). A single ewe, used in Trial 1, received an inoculum of 2.5 ml of culture of *L. pomona* (strain 3050) subcutaneously. Strain 3050 was originally isolated from sheep. Rectal temperatures were recorded daily through postexposure day 7. Hemocultures were prepared on postexposure days 5 to 7 inclusive, incubated at 28°C and examined by darkfield illumination (525x) at weekly intervals for one month. Serum titer was checked by the agglutination-lysis test. Seventy and 73 days post exposure the ewe was given 4.5 ml of disrupted cell suspension. In Trial 2, 3 ewes were inoculated with strain 3050 and one ewe received 2.5 ml of sterile isotonic saline. Postexposure procedures were the same as in Trial 1 until day 15. All 4 ewes were given 4.5 ml of disrupted cell preparation on postexposure day 15. On day 19, ewe 41 received 1½ ml and the other 3 ewes 3 ml intravenously. Hemoglobin values were determined as cyanmethemoglobin by optical density with a Bausch and Lomb Spectronic 20 colorimeter at 540 mμ. Hematocrit values were ascertained in Wintrobe hematocrit tubes.

Results. The exposure to *L pomona* (strain 3050) in Trial 1 produced a mild leptospirosis demonstrated by recovery of the organism in hemocultures and agglutination-lysis reactions in serum dilutions of 10^{-3}. Hemoglobinuria was observed 48 hours after subsequent administration of the cell suspension, and persisted until the ewe died approximately 60 hours later. Ten hours before death the hemoglobin value was 2.7 g per 100 ml of blood and packed cell volume 7.0%. The terminal agglutination-lysis reaction was 50% complete in a serum dilution of 10^{-4}.

In Trial 2, leptospirae were isolated in hemocultures from 2 of 3 exposed ewes. Agglutination-lysis reactions were observed in serum dilutions of 10^{-2} on postexposure day 9. Maximal rectal temperatures observed from postexposure days 5 to 9 were 102.2 to 104.4. The control remained normal. On postexposure day 17, two days after intravenous injection of the cell suspension, hemoglobinuria was observed in ewes 33, 37, and 41 but not in the non-immune control (Fig. 1,2,3,4). Hemoglubinaria persisted for at least 24 hours and was associated with a drop in hemoglobin of 2.4 to 3.9 g per 100 ml of blood. Hemoglobin values stabilized and the hemoglobinuria became less marked. The control ewe developed detectable leptospiral antibodies 48 hours after receiving the cell suspension. Reinoculation with 3 ml of the cell suspension on day 19 was followed by hemoglobinuria in ewes 33, 37 and control. Hemoglobinuria was transient in ewe 33, less than 12 hours duration, with a concurrent drop in hemoglobin of 1.4 g. Ewe 37 had marked hemoglobinuria for 36 hours accompanied by a loss of 3.1 g of hemoglobin. Hemoglobinuria was first observed in the control 48 hours after the second exposure to the cell suspension with a loss of 4 g of hemoglobin per 100 ml in a 96 hour period. Ewe 41 received one half the amount of cell suspension on reinoculation and no hemoglobinuria resulted. The agglutination-lysis titer decreased following inoculation with the cell suspension in those instances where

hemoglobinuria was observed.

Discussion. Hemoglobinemia, frequently accompanied by hemoglobinuria, is a frequent manifestation of experimental *L. pomona* infections in sheep and cattle. The pathogenicity of *L. pomona* has been attributed to the cell-free supernatant fluids of cultures. In referring to the hemolytic factor described by Alexander *et. al.*(4) Alston and Broom(12) state, "The authors suggested that the presence in leptospires of a soluble hemolytic agent may be a significant factor in determining the pathogenicity of leptospiral infections. This is a tempting speculation, but the evidence at present available does not appear to favour it." Morse *et. al.* (3) reported hemoglobinuria in 5 experimentally infected lambs. Antibodies were detected in the serum of 4 of these lambs prior to or concurrent with onset of hemoglobinuria and while circulating antibodies were present. The premise was established that, with large numbers of leptospirae present in the blood, antibody actively lyses the leptospirae releasing a hemolytic endotoxin capable of lysing red cells in presence of specific antibody and complement.

The hemoglobinuria observed in this experiment appeared to be an antibody requiring phenomenon. The control ewe did not evidence hemoglobinuria or marked hemoglobinemia on the first exposure to 3 ml of the cell suspension. A second administration of cell suspension subsequent to the appearance of circulating antibodies resulted in hemoglobinuria, suggesting that specific antibodies were required. Hemoglobinuria was usually associated with a drop in serum titer of the magnitude of one log, 24 hours after administration of the cell suspension. The drop in titer could be attributed to introduction of the specific antigen with an antibody-antigen reaction following, essentially an *in vivo* absorption phenomenon. The requirement for circulating antibody to produce hemolysis gives credence to the premise of a specific requirement for antibody plus a component of the cell suspension. Antiglobulin tests should be applied to determine the relationships between the components of the system.

Amount of cell suspension administered was directly related to degree of hemolysis observed. The ewe in Trial 1 had severe hemoglobinemia and hemoglobinuria. A second administration of 4½ ml of cell suspension injected during hemoglobinuria resulted in continued hemolysis and eventual death, attributed to the severe anemia. In Trial 2, 3 ml of cell suspension produced hemoglobinuria in immune animals while 1½ ml did not. Hemoglobin is a threshold substance and hemoglobinuria does not occur until plasma concentration reaches 150 mg%(13). Administration of 1½ ml of cell suspension did not result in hemolysis of a magnitude that would produce such a plasma concentration.

The results of this experiment suggest an antibody requiring cellular hemolytic factor, probably an endotoxin, that manifests itself in a manner that closely parallels that observed in natural or experimental infection with *L. pomona*. The unidentified factor in the cell suspension has a greater propensity as a virulence factor involved in the pathogenicity of *L. pomona* infection than the factor demonstrated in the cell-free culture supernatant fluids.

Summary. Hemoglobinuria and hemoglobinemia in ewes followed intravenous injection of disrupted *L. pomona cells*. Hemolysis occurred only in presence of antibody. It is postulated that the hemolytic effect is due to interaction of a cellular component, antibody and red blood cells.

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Effect of Gamma Irradiation and AET on Rat Blood Cholinesterase.* (26416)

MARTIN W. WILLIAMS, ROGER D. BAKER AND R. WADE COVILL
Department of Pharmacology, University of Vermont College of Medicine, Burlington

Previous work by Sabin(1) has indicated that red blood cell cholinesterase levels of the mouse offer a sensitive biological index to x-irradiation. Williams(2), by a different technic, has shown similar action of gamma irradiation in whole blood cholinesterase of the rat, but the threshold by this index was not determined in this species. In the same study the acute toxicity of acetylcholine was shown to be greater in gamma irradiated mice than in the non-irradiated animals.

With the discovery of Patt, *et al.*(3), that cysteine and glutathion gave radiation protection, further studies by Bach and Herve (4) on radio-protective agents and later work by Shapira, *et al.*(5) led to development of several radio-protective materials. One of these, S, 2-aminoethylisothiouronium bromide, hydrobromide (AET) has been studied rather extensively and was utilized in this investigation.

The purpose of this study was 2-fold: 1) to determine the irradiation threshold for whole blood cholinesterase activity in the rat and to plot a dose response curve of these data; 2) to determine whether AET produces any *in vivo* gamma irradiation protective action insofar as whole blood cholinesterase activity is concerned.

Methods. Rats utilized were irradiated by whole body exposure in lucite boxes to a cobalt-60 source of 820 curies at a source to midline distance of 150 cm. Dosimetry was accomplished utilizing the Victoreen model 70 r-meter and hi-energy chamber model 620. Exposure rate at this distance was 9.37 r per minute.

Dosage levels tested were 0, 75, 150 and 300 r except where otherwise noted. The 10 animals per level utilized were male Wistar strain Charles River rats, weighing 100 to 135 g at beginning of experiment. Cholinesterase determinations were by a micro technique similar to that of Michel(6) and involved the use of one-tenth ml of whole blood. The blood was drawn by tail vein into a one-tenth ml pipette and transferred to a 15 ml centrifuge tube containing 2 ml of distilled water as a hemolyzing agent. The fluid level in the tube was then brought up to 2.5 ml with distilled water and then to 5 ml with a buffer containing 0.82 g sodium diethyl barbiturate, 0.10 g of potassium (monobasic) phosphate and 44.70 g of potassium chloride per liter. The pH of this buffer was adjusted to 8.3 with 0.10 M hydrochloric acid, prior to use. The centrifuge tubes were then placed in a water bath at 37° ± 0.5°C and 0.5 ml of 3 per cent acetylcholine bromide was added.

The initial pH was then read and incubation continued for 2 hours after which the final pH was recorded. The difference between these values was recorded as the delta pH. Non-enzymatic hydrolysis of the acetylcholine was virtually non-existent so no correction was made. Mean initial control whole blood cholinesterase activities, i.e. delta pH, under these conditions were 1.04 pH units per 2-hour incubation period.

All animals used were given Purina Lab Chow and water *ad libitum* and were housed

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