likelihood that molybdenum poisoning will ever be a practical problem with poultry.

Summary. Growth of chicks and poults was depressed by approximately 25% by the addition of 300 p.p.m. molybdenum to their rations. The addition of copper to the rations containing molybdenum caused slightly improved growth. There was no evidence of anemia or diarrhea in birds on the high molybdenum diets. Poults fed high molybdenum rations for over 4 weeks had normal feather pigmentation.

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A Method for Quantitative Identification of Canine Erythrocytes. (19665)

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(Introduced by Theodore E. Friedemann.)
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Studies of erythrocyte metabolism and function in the dog have been hindered by the lack of a reliable quantitative method of red cell identification. Hamilton(1) and Young *et al.*(2) have recently alluded to serologic, and Harrison *et al.*(3) to isotopic technics. In the present communication, we report a method for the quantitative identification of canine erythrocytes by differential agglutination utilizing lyophilized anti-sera. Young and his collaborators(2,4) have described 6 erythrocyte agglutinogens in dogs. These have been arbitrarily designated as A, B, C, D, E, and F. These canine agglutinogens do not correspond to human agglutinogens of similar terminology. In random examination of a large group of dogs, the A agglutinogen was found in about 63% of dogs, the B in about 5%, the C in 99%, the D in about 30% and the E in 80%. The incidence of the F agglutinogen has not been reported. Presumably there are other agglutinogens not yet identified. Christian *et al.*(4) have observed that variants of the A agglutinogen exist. These have been designated A′ and are present in about 40% of A dogs. The A-A′ differences are partly qualitative and are thought to resemble human D-D^u differences more closely than human A_1-A_2 differences.

Isohemagglutinins have been demonstrated in dog sera. Anti-D has been observed as a naturally occurring antibody, while anti-A,-B,-C,-E, and -F have been demonstrated only in dogs transfused with erythrocytes containing the respective factors.

Methods. The A agglutinogen is strongly antigenic, and high titre anti-A sera can be produced by multiple transfusions of A cells into dogs whose erythrocytes do not contain the A agglutinogen. However, at the same time there is produced an hemolysin which is not specific for A cells. It was found that

TABLE I. Survival of Transfused Erythrocytes in a Normal Dog.

Day of study	Blood vol, ml	Plasma vol, ml	Circulating red cell mass, ml	Recipient cells, ml	Infused cells, ml
			Before infusion		
20	1220	705	515	515	—
16	1200	720	480	480	—
1	1285	770	515	515	—
Mean	1235	732	503	503	—
			After infusion		
0	1246	732	516	427	89
5	1270	750	520	436	84
17	1285	770	515	448	67
35	1300	750	550	500	50
38	1310	730	580	534	46
69	1256	715	540	517	23

this hemolysin could be removed by absorption with appropriate red cells. In our red cell survival studies, it was decided to use only C, D, or CD dogs as donors and as recipients, AC, AD, and ACD dogs (with or without E and F agglutinogens). Thus, no known iso-antibodies would be developed to the transfused cells. To remove the hemolysin, fresh CD red cells which have been washed 3 times in isotonic sodium chloride solution were added to anti-A serum (titre 1:128 or greater) in which complement had been inactivated by incubation for one-half hour at 56°C. The mixture was kept at room temperature (about 25°C) for one-half hour. The serum was then removed after centrifugation. The addition of washed CD cells (saline, in amount about 10 times the volumes of the red cells, was used for each washing) was repeated until there was no hemolysis of CD cells. Usually 3 additions of CD cells were sufficient to absorb the hemolysin. Hemolysis was detected as follows: An autologous serum suspension of CD cells was made; one side of a counting chamber was filled with this mixture. At the same time 0.1 ml of the suspension was added to 0.1 ml CD absorbed anti-A serum in a 10 x 75 mm tube. This second mixture was allowed to stand at a temperature of about 25°C for 15 minutes and was then centrifuged for 3 minutes at 1000 RPM. The tube was shaken vigorously against the palm of the hand to free the cells, and the other side of a counting chamber was flooded with a sample of the supernatant mixture. The entire red cell field was counted on both sides of the chamber. In the absence of hemolysis, the count in the first chamber was twice that in the second chamber. After anti-A sera had been adequately absorbed with CD cells, they were shell-frozen at −70°C and lyophilized. The dried sera were pulverized and mixed into an homogenous mixture and stored in a desiccator over calcium chloride at 4°C. To reduce the quantity of blood required for serial observations of survival of transfused red cells, it was decided not to use autologous sera for making red cell suspensions for differential agglutination measurements. Isotonic saline as a suspending medium was not suitable since agglutination under these conditions was incomplete and free counts of 20 to 25% were obtained. Bovine albumin (10 to 20% in saline) resulted in excessive rouleaux formation and incomplete agglutination (free counts of 8 to 12%). Homologous serum from dogs with the same red cell types as the recipient dogs proved satisfactory as a suspending fluid. Free counts were negligible, and *in vitro* mixtures of red cells could be counted within an error of about 5%.

The differential agglutination counting technic is as follows: Oxalated blood from a recipient dog (or an *in vitro* mixture) is taken in a red cell pipette to the 0.5 mark and suspended in one ml fresh serum from an ACD dog. A total count is done on this suspension and, from the same pipette, 3 to 4 drops of the cell suspension is added to a 10 x 75 mm test tube in which dried absorbed anti-A serum has been placed. Enough of the

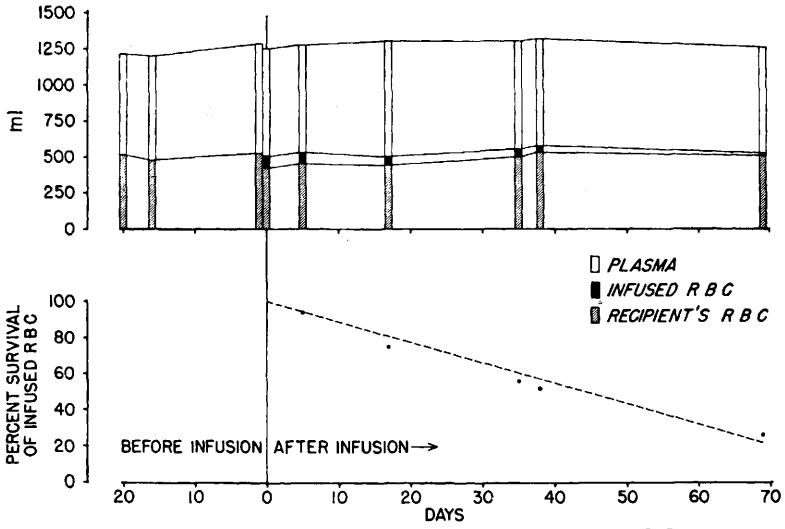

FIG. 1. Survival of transfused erythrocytes in a normal dog.

dried serum is used to cover the bottom of the test tube to a height of about 1/16 of an inch. No significant change in volume of the added cell suspension occurs. The tube is allowed to stand for 15 minutes at a temperature of about 25°C. It is then centrifuged for 3 minutes at 1000 RPM. The tube is shaken against the palm of the hand to free the cells and sufficient chambers are filled to enable the counting of 1000 unagglutinated cells. The counting is done immediately; a delay of over thirty minutes leads to non-specific hemolysis.

Experimental. The survival of infused normal canine erythrocytes was studied in four healthy mongrel dogs maintained on a constant nutritionally adequate diet. Serial measurements of nitrogen intake, urinary and fecal nitrogen output, various liver functions (serum bilirubin, bromsulfalein retention, prothrombin time, and thymol turbidity), iliac crest bone marrow morphology, and the peripheral red and white cells were made. Plasma volumes were measured with T-1824 (5) and the circulating red cell masses calculated(6). After a series of control observations, approximately 20% of the blood of the recipient dog was withdrawn and immediately replaced with an equal quantity of blood just withdrawn into acid-citrate-dextrose from the donor dog. Replacement transfusions were carried out to minimize effects of the procedure on erythropoiesis(7,8). Serial measurements of plasma volumes, and red cell masses, donor and recipient, were made. These data of a representative study are presented in Table I and Fig. 1. The destruction of the infused cells proceeded at a rate of about 1% per day. This is similar to the figures mentioned by Young *et al.*(2), Hamilton(1) and Harrison *et al.*(3). No changes in bone marrow morphology or peripheral leucocytes were noted. Nitrogen balance was not significantly changed.

Summary. A procedure for the quantitative determination of injected canine erythrocytes by a differential agglutination technic utilizing lyophilized type-specific anti-sera is described. The method was applied in a study of erythrocyte survival after transfusion of blood. Approximately 20% of the blood of the recipient dogs was withdrawn and immediately replaced with an equal volume of donor blood. Determinations were made of red cell volume, plasma volume, donor and recipient red cell counts. Serial examinations of bone marrow and peripheral leucocytes were also made. Destruction of infused cells proceeded at a rate of about one per cent per day. No changes in bone marrow morphology or peripheral leucocytes were noted. Nitrogen balance was not significantly changed.

The opinions are those of the authors and do not necessarily represent the official views of any governmental agency.

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Comparative Effectiveness of Five Newly Synthesized Arsenical Compounds in Treatment of Leukemia in Mice. (19666)

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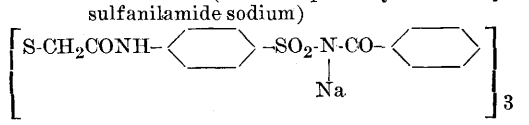
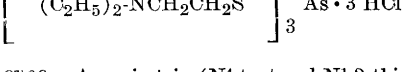
Arsenic in the form of potassium arsenite (Fowler's solution) is an effective therapeutic agent in chronic myelogenous leukemia. Theoretically the arsenic acts as a general poison by combining with the thiol compounds present in protoplasm, probably SH components of the pyruvate oxidase enzyme system(1). In chronic myelogenous leukemia there seems to be a selective action on the myeloid cells(2). It is our impression that this selective action of Fowler's solution on myeloid elements is greater than that of other agents such as urethane and irradiation so that fewer instances of concurrent depression of the other marrow elements occur. It therefore seemed desirable to study the effects of other arsenical compounds in an effort to find one of greater therapeutic efficiency. In this study 5 newly synthesized trivalent organic arsenical compounds (with the arsenic not attached to a benzene ring) were compared along with potassium arsenite for their ability to prolong the lives of mice affected with transmitted leukemia. In planning the types of organic arsenic compounds to be tested, it seemed desirable to so modify the organic "carrier radical" that transport across cell membranes might significantly differ with each type. The identifying MC numbers, structures and names of the compounds tested are shown below:

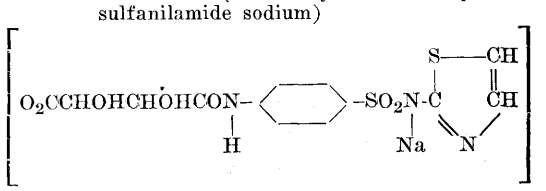
MC 2186—Arsenic-tris-(N^4-mercaptoacetyl-N^1-benzoyl sulfanilamide sodium)



MC 2767—Tris-(β-Diethylaminoethylthio)arsine hydrochloride

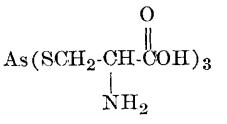


MC 2768—Arsenic-tris-(N^4-tartaryl-N^1-2-thiazolyl-sulfanilamide sodium)



MC 2864—Tris (β-hydroxyethylthio) arsine $(HOCH_2CH_2S)_3$ As

MC 2865—Tris (β-carboxy-β-aminoethylthio) arsine



In Type 1 (MC 2186 and 2865), arsenic is bound to sulfur as part of an anion; in Type 2 (MC 2767), arsenic is bound to sulfur as part of a cation; in Type 3 (MC 2864), arsenic is bound to sulfur in a neutral molecule;