rate, support the assumption that the changes are due to myocardial depression. The most logical explanation for the depression of contractile force and heart rate below a mean arterial pressure of 90 mm Hg is that this represents a critical coronary perfusion pressure. With profound hypotension the role of coronary insufficiency in the depression of ventricular function has been previously shown(11), but the present results indicate that neither prolonged nor severe circulatory depression is necessary for the occurrence of myocardial depression in response to hemorrhage. In a study of dogs with totally denervated hearts* neither augmentation nor depression of heart rate and myocardial contractile force occurred in response to acute hemorrhage. These findings suggest that in addition neurogenic factors influence the myocardial response to rapid blood loss.

Summary. The changes in myocardial contractile force and heart rate which accompanied acute bleeding were studied in normal dogs. Increases in contractile force and heart rate were noted in response to hemorrhage until mean arterial pressure fell to less than 90 mm Hg. Below this level of pressure depression of both modalities began and continued as pressure was further lowered. These findings indicate that myocardial depression occurs at an early stage in acute bleeding and probably results from a critical diminution in coronary perfusion pressure.

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Partial Purification of Cattle Serum Transferrin Using Rivanol.* (26779)

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Chemical studies of genetic differences in proteins are invaluable in understanding the mechanism of gene action. Inherited variations in the serum proteins of many animal species can be used to study gene-controlled protein specificity(1). Transferrin, also known as siderophilin or β-metal combining globulin, appears to be a very promising subject for such a study, since inherited variations in this protein have been demonstrated in many animal species(2-5), and much is known about its chemical properties(6).

Transferrin of cattle serum can be resolved into at least 4 components by starch gel electrophoresis(7,8). The mobility of all of these are affected by a change in the transferrin locus. Five alleles have been described so far, designated β^A, β^B, β^D, β^F, β^E, in order of decreasing mobility of the corresponding transferrin(9). Different breeds of cat-

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tle have different frequencies of these alleles (9,10).

Transferrin is usually isolated by fractionating serum with ethanol or ammonium sulfate(11-13). Boettcher *et al.*(14) have devised a simpler approach. Rivanol (2-ethoxy 6,9-diaminoacridine lactate) is added directly to human serum to precipitate albumin and most of the α-globulin fraction. Transferrin has been purified and crystallized from this supernatant(15). In our work, transferrin from cattle serum has been partially purified using rivanol. Our ultimate goal is to prepare cattle transferrin pure enough for chemical studies of the phenotypic differences. In addition, we wish to determine the nature of the apparent multiplicity of this protein.

Materials and methods. Rivanol was obtained from the Special Chemical Department of Winthrop Laboratories under the trade name "Ethodin." Fractionation was carried out as follows: Serum was saturated with respect to iron by addition of 12 μg iron (as ferrous ammonium sulfate) per ml serum. The mixture was kept at 5°C for one hour. A predetermined volume (see below) of 0.4% aqueous rivanol was then added to one volume serum. The final pH was adjusted to 8.5-8.6 with 0.1 *N* sodium hydroxide and the mixture kept at 5°C for one hour. The precipitate was removed by low speed centrifugation, and dissolved in 0.1 M acetate buffer, pH 4.7. The supernatant was treated with Norite or dialyzed to remove rivanol.

Electrophoresis of all fractions was carried out in vertical starch gels(16) using borate buffer, pH 8.6. Some of the fractions were also analyzed by paper electrophoresis using an E-C pressure-plate electrophoresis apparatus. The buffer used contained 58.8 g sodium barbital, 23.4 g anhydrous sodium acetate, 3 l distilled water, and 2.5 ml glacial acetic acid. The final pH was 8.6. After electrophoresis in starch gel or on paper, the proteins were stained with Amido Black. Six μl serum were used for analysis on paper and about 0.1 ml for starch gel.

Results. One-volume samples of serum were treated with increasing volumes of 0.4% rivanol to determine the ratio of rivanol to serum that would give maximum purification of transferrin. After addition of the appropriate amount of rivanol, the precipitates were removed by centrifugation and the supernatants analyzed by starch gel electrophoresis. The rivanol migrated towards the cathode and off the gel completely. No correction was made for the dilution effect of the rivanol solution added. The results of this first experiment are shown in Fig. 1. Addition of an equal volume of 0.4% rivanol to serum did not affect the pattern on starch gel appreciably, while a 2:1 ratio of rivanol to serum removed a large amount, but not all, of the albumin and some of the slow α-globulin. At a ratio of 3:1 there was no albumin and very little if any slow α-globulin visible on the gel. At this ratio, the transferrin bands were clearly visible and apparently unaffected by the rivanol treatment, as compared to the untreated serum. At ratios above 3:1, the transferrin bands became faint, but were still clearly visible. From these results it was concluded that the optimum ratio of rivanol to serum was 3:1, and this ratio was used in all further experiments.

In the next experiment, serum from the same cow, (BF 486, Holstein-Fresian Breed, type β^D/β^D) was treated with rivanol, and after removal of the rivanol, the supernatant was lyophilized and redissolved in distilled water to the same volume as the original serum. Likewise, the precipitate was reconstituted using 0.1 *M* acetate buffer at pH 4.7. A considerable amount fo protein in this fraction failed to dissolve so the sample used for electrophoresis was heterogeneous. Only transferrin and γ-globulin were clearly visible in the supernatant fraction. As expected, the insoluble fraction contained albumin and slow α-globulin, but only trace amounts of transferrin and γ-globulin.

The fractions of serum obtained after treatment with rivanol were studied also by paper electrophoresis. The insoluble fraction contained considerable amounts of β-globulin, even though only a small amount of transferrin was present. Also, a considerable amount of insoluble protein remained at the origin in this fraction. The evidence from both paper and starch gel electrophoresis in-

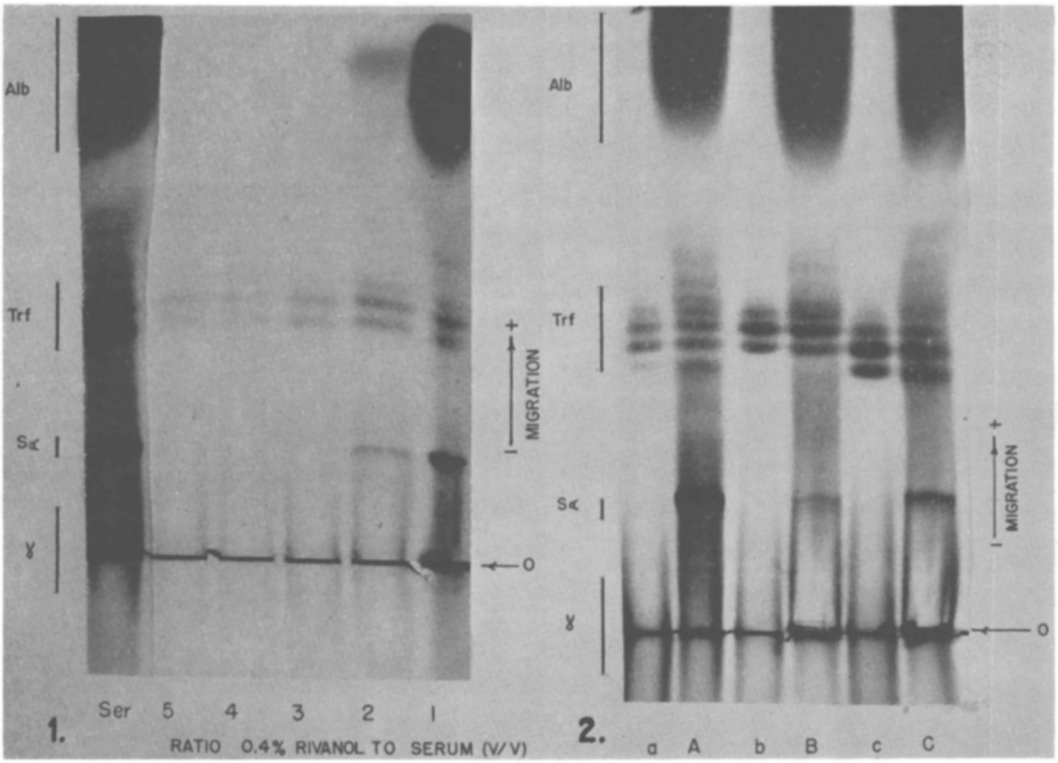

FIG. 1. Starch gel electrophoresis patterns of fractions of cattle serum after addition of varying amounts of rivanol solution. Ser = untreated serum. Samples 1-5 = soluble fractions after addition of 1-5 volumes of 0.4% rivanol to one volume serum. (Cow BF486, type β^D/β^D.) O = origin; γ = γ-globulin; Sα = slow α-globulin; Trf = transferrin; Alb = albumin.

FIG. 2. Starch gel electrophoresis patterns of serum from cows of 3 different transferrin phenotypes and corresponding supernatant fractions of sera after treatment with 0.4% rivanol. A = serum of type β^A/β^D; a = supernatant fraction of A; B = serum of type β^A/β^A; b = supernatant fraction of B; C = serum of type β^D/β^D; c = supernatant fraction of C. Other symbols as in Fig. 1.

dicates that treatment of cattle serum with 0.4% rivanol removes albumin and the α-globulin fraction.

Serum from each of 3 cows with phenotypically distinct transferrin types was treated with rivanol to see if the transferrin behaved similarly. As shown in Fig. 2, the supernatant fractions of all 3 cattle sera contained transferrin with the same patterns and mobilities as the untreated sera.

The genetically controlled variants in the serum β-globulin of humans and cattle were first shown to be transferrin by autoradiographs using iron-59 (3). We have confirmed this for cattle using our partially purified transferrin preparations.

Discussion. Our results with rivanol fractionation of cattle serum closely parallel those of other workers using human serum (14,17). At ratios of 3:1 (v/v) 0.4% rivanol to serum, the albumin and α-globulin fractions of cattle serum are almost completely precipitated, leaving transferrin and γ-globulin in solution. Although cattle transferrin can be resolved into at least 4 bands by starch gel electrophoresis, treatment of serum with rivanol does not differentially affect the bands. In this respect, transferrin behaves as a unit.

Nothing is as yet known about the chemical basis of the differences observed between individuals of different transferrin phenotypes. Differences in mobility have been demonstrated by paper electrophoresis for the products of different transferrin alleles in both human and mouse serum (2,4). Human and porcine transferrin have been crystallized and found to contain considerable amounts

of sialic acid and hexosamine(6), so that the genetically controlled differences may involve either amino acids or carbohydrates. The nature of the multiplicity of transferrin from cows homozygous at the transferrin locus also remains an unsolved problem. It is possible that the bands are composed of the same protein with different numbers of sialic acid moieties attached. It has been found that treatment of human transferrin with neuraminidase results in the appearance of 5 bands whose intensity varies with the concentration of the enzyme(18,19). Direct evidence bearing on these questions, however, must await further purification of these proteins.

Summary. A ratio of 3 volumes 0.4% rivanol to one volume serum was used to partially purify cattle transferrin. After treatment, the supernatant fraction contained predominantly transferrin and γ-globulin. No albumin was detectable by starch gel or paper electrophoresis. Fractionation of serum from cattle of phenotypically different transferrin types yielded transferrin with the same pattern and mobility in starch gel electrophoresis as in the original serum.

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Pharmacological Studies with Polythiazide, a New Diuretic and Antihypertensive Agent.* (26780)

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The search for orally effective and safer diuretic agents led to the introduction of benzthiadiazines as a new class of diuretics (1,2).

Recently attempts have been made to develop more potent benzthiadiazines with a

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