was the EAR positive or was there a significant deviation from the normal electrophoretic pattern.

Summary. The production of tissue necrosis by various aseptic techniques induced a positive erythroagglutination reaction in non-tumor-bearing golden hamsters within 6 days. The rapidity of onset and the duration of the EAR varied somewhat, depending on the procedure, but there was no associated characteristic change in the serum protein profile, and anemia appeared in only 2 animals. Freund's adjuvant induced a positive EAR in all animals within 6 days, but very little change in the serum protein profiles.

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Production of Members of the Blood Coagulation and Fibrinolysin Systems by the Isolated Perfused Liver.* (29161)

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Whipple and Madden(1,2) reviewed the clinical and experimental evidence indicating that most plasma proteins, including fibrinogen, are produced by the liver. Direct demonstration was accomplished only later(3-9) using radioisotopes and immunologic methods in experiments with isolated livers or liver slices. More detailed study of individual clotting factors were undertaken by Pool and Robinson(10) who found that rat liver slices *in vitro* produce factor VII and to a lesser extent, prothrombin. Barnhart(11) identified the liver as the source of prothrombin by immunofluorescent methods. The purpose of this study was to investigate the production of members of the blood coagulation and fibrinolysin systems by the isolated liver.

Materials and methods. The apparatus and procedure to maintain isolated perfused rat livers was that of Miller *et al*(6,7) as modified by Aydin and Sokal(12). All glassware was siliconized. Sprague-Dawley rats of about 250 g body weight were given 10 mg vitamin K intramuscularly; 24 hours later they were anesthetized with ether and the isolated liver perfusion established. The bile duct was cannulated and bile flow was used as one of the criteria of viability of the preparation. About 25 ml of perfusing medium was allowed to pass through the liver and the effluent discarded before recirculation of the medium was established. A blood sample taken at this time was used to establish baseline values of the blood coagulation and fibrinolysin systems. The perfusing medium was prepared by bleeding 15-20 Sprague-Dawley rats under ether anesthesia through the aorta, using a 19-gauge Fenwall hemorepellent needle and a 10 ml siliconized glass syringe containing 1 ml of 3.8% sodium citrate solution. The pooled blood (200 ml) was centrifuged at 1500 rpm in a refrigerated centrifuge (4°C) for 10 minutes, the platelet rich plasma and buffy coat were discarded and the red cells were washed 3 times with citrated saline (100 ml of 3.8% sodium citrate, 500 ml of 0.85% sodium chloride) solution. The red cells were then suspended in an equal volume of medium made up as follows: 100 ml Tissue Culture Medium 199

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(Difco Laboratories), 10 ml citrated saline, 22 ml 25% human serum albumin (Albumisol, Merck, Sharp & Dohme Co.), 0.1 ml (1 mg) vitamin K (Konakion injectable, Hoffmann-LaRoche Co.), 2 mg Adenosine-5′-Triphosphate.

During the experiment 9 ml samples were withdrawn periodically from the blood reservoir using hemorepellent needles and siliconized glassware. Red cells were removed by centrifugation and the supernate analyzed. Factors II(13), V(14) and VII-X(13) were determined by using the samples to correct the faulty clotting times obtained with Simplastin (Warner-Chilcott Co.) of specifically deficient substrates. Results were compared to those obtained with serial dilutions of normal rat plasma and expressed as percentages of the normal values. The method of Owren and Aas(13) determines a composite of factors VII and X without differentiating their relative level. The thromboplastin generation test (TGT)(15) was modified to use Inosithin (Associated Concentrates Co.) as a platelet substitute and $BaSO_4$-adsorbed oxalated plasma. The samples were diluted 1:5 and used as plasma reagents (without adsorption) with a 1:10 dilution of normal human serum. Also, 1:10 dilutions of the samples were used as serum reagents with a 1:5 dilution of oxalated $BaSO_4$-adsorbed normal human plasma. Members of the fibrinolysin system (plasminogen activators, proactivators, plasminogen, plasmin, antiplasmins) were determined by methods published earlier (16-18). Results were expressed in RPMI units/ml(16,17).

Rat liver slices of 2-3 mm thickness were prepared from freshly sacrificed animals using an A. H. Thomas Co. slicer. Slices from 6 rats were rinsed in 6 changes of citrated saline, pooled, and distributed into six 50 ml beakers. Each slice was separated from the next by a square of neoprene open mesh matting. Twenty ml of perfusing medium (see above) was added to each beaker. The beakers were gently rotated in a Brunswick Gyratory Shaker-Incubator equipped with a gassing hood, at 37°C, and gassed with a mixture of 95% O_2 and 5% CO_2. Aliquots were withdrawn and analyzed as with the isolated perfused liver preparations.

Results and discussion. Fig. 1 shows the appearance of factors VII-X in the perfusing medium in 10 experiments. There is a linear increase in the levels of these factors and in 8 hours, about one-quarter of the normal factor VII-X content of rat plasma is reconstituted. Fig. 2 shows that prothrombin is produced and/or released into the medium at a much lower rate than factors VII-X, and that its level reaches a plateau after about 6 hours. These results are in agreement with the findings of Pool and Robinson(10), who reported that liver slices *in vitro* released factor VII, but not prothrombin, to the medium and that a lesser production of prothrombin could be demonstrated only by analyzing the tissue. Fig. 3 depicts production of factor V; a plateau is obtained at about 4 hours and total production is less than that of factors VII-X. Fig. 4 shows production of antiplasmin in the same system. No definite plateau is obtained. The same Figure also shows the results of a single experiment with liver slices in which the antiplasmin level was measured in the incubation medium. Antiplasmin release was quite similar to that observed with the isolated perfused liver during the first 8 hours, and continued for 48 hours. Antiplasmin values are expressed in RPMI units/ml rather than as percentages of the normal level. Normal rat plasma contains about 14-40 RPMI units of antiplasmin/ml. The isolated perfused liver reconstituted about 15-20% of the normal antiplasmin level in the perfusing medium in 8 hours. In the medium bathing liver slices, 15-50% of the normal values were obtained in 48 hours. No other fibrinolytic factors (plasminogen, plasminogen activators, proactivators, plasmin) could be demonstrated, either in the liver perfusion medium or the slice incubation medium. No thromboplastin generation was evident with the medium acting as either plasma or serum reagent. It was felt that the relative insensitivity of the available specific assays for trace amounts of factors VIII, IX, XI, and XII would preclude employment in this study.

Summary. The isolated liver of rats pretreated with vitamin K_1, perfused with red

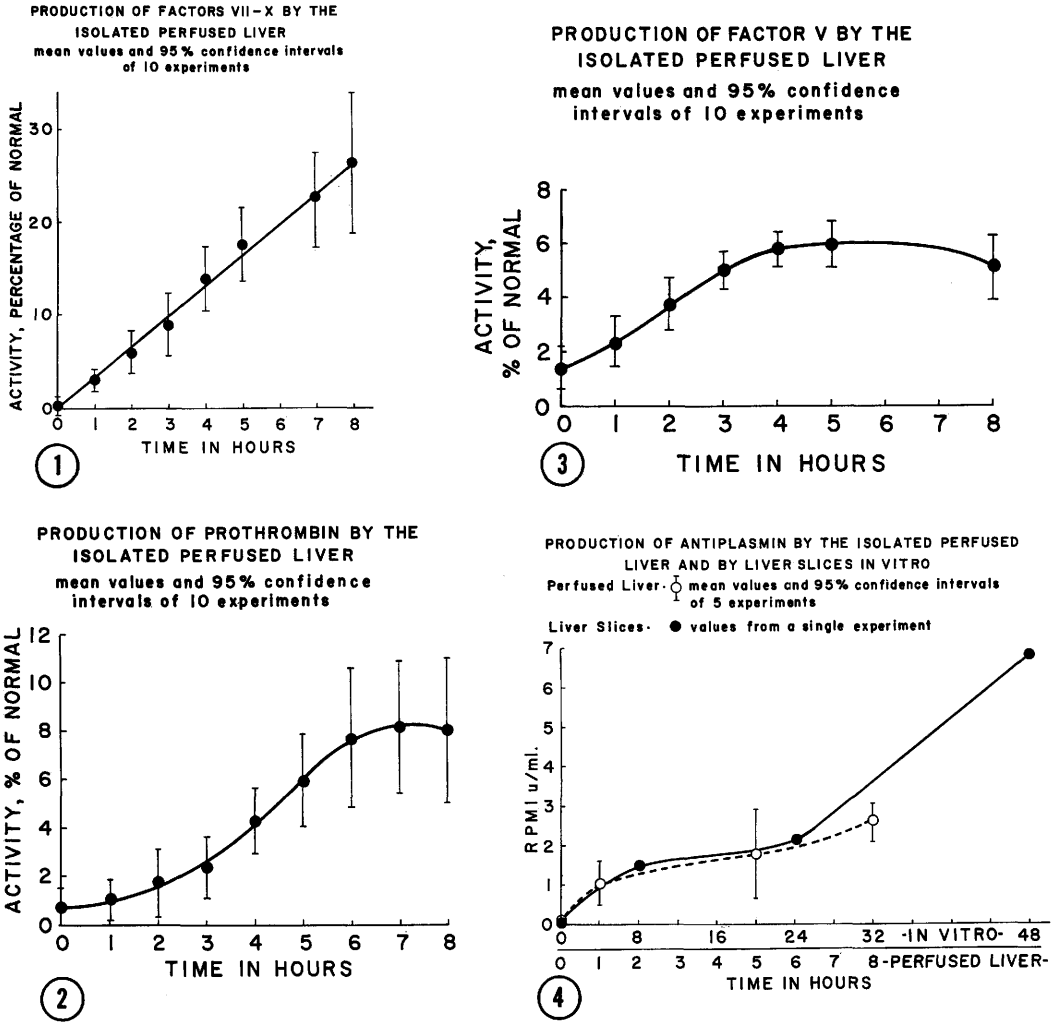

FIG. 1-4

cells suspended in a nutrient medium devoid of blood coagulation components, produced coagulation factors VII-X (stable factor, proconverten-convertin and Stuart factor), factor V (labile factor, proaccelerin) and factor II (prothrombin). No thromboplastin generation was observed. No production of plasminogen activators, proactivators, plasminogen and plasmin could be detected in any of the experiments, but antiplasmin release was observed from the isolated perfused rat livers and in the nutrient medium of rat liver slices incubated *in vitro*.

Addendum. After submission of this manuscript an abstract by Olson, Miller and Troup came to our attention (Abst., 6th Ann. Meeting Am. Soc. Hematol., 1963) which described production of factors II, VII, X and fibrinogen by the isolated rat liver. Components of the fibrinolysin system were not studied.

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Conversion of Acetyl Strophanthidin-induced Ventricular Tachycardia to Sinus Rhythm by Isopropyl Methoxamine.* (29162)

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Digitalis toxicity is manifested by a multiplicity of changes in the electrocardiogram, usually terminating in ventricular tachycardia and ventricular fibrillation. Clinically, such drugs as quinidine, procaine amide and potassium are used to treat ventricular arrhythmias caused by digitalis but these drugs are not always effective and many times prove dangerous in themselves. Thus, the search continues for other drugs to treat digitalis toxicity. This paper is a report of the drug isopropyl methoxamine, found to be effective in the conversion of acetyl strophanthidin-induced ventricular tachycardia to sinus rhythm.

Acetyl strophanthidin,† a synthetic ester of the aglycone strophanthidin, was chosen because of its very rapid action. Pharmacologic and clinical studies have revealed that acetyl strophanthidin produced essentially the same effects as those of digitalis glycosides(1-4).

Methods. Mongrel dogs weighing between 8 and 12 kg were anesthetized with 30-35 mg/kg pentobarbital sodium intravenously or intraperitoneally. They were intubated with a cuffed Portex endotracheal tube under direct vision and ventilated with 100% oxygen at a tidal volume of 250 ml and at a rate which varied from 12 to 20 per minute with a Harvard respirator pump, depending upon the minute volume required to maintain controlled respiration. Arterial blood pressure, electrocardiogram, lead 2, and a right atrial lead were monitored continuously at a paper speed of 25 mm/min. At intervals, short runs of 25 mm/sec were taken in order to interpret the electrocardiogram properly. Acetyl strophanthidin was infused at a constant rate of 6 μg/kg/min in 9 dogs. Within 8 minutes of the onset of ventricular-initiated beats, isopropyl methoxamine‡ was injected intravenously at various dose levels. In all experiments the acetyl strophanthidin was infused until death of the animal occurred as evidenced by absence of blood pressure. In 4 experiments blood samples were taken for analysis of serum potassium.

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† Acetyl strophanthidin was kindly supplied by Eli Lilly Co., Indianapolis, Ind.

‡ BW 61-43 or *dl*-erythro-α-(2,5-dimethoxyphenyl)-β-isopropylaminopropanol hydrochloride kindly supplied by Burroughs Wellcome & Co., Tuckahoe, N. Y.