Effects in Patients of Intravenous Infusions of Purified Streptokinase Preparations.* (22913)

A. J. JOHNSON, A. P. FLETCHER, W. R. MCCARTY, AND W. S. TILLETT
Department of Medicine and Surgery, New York University College of Medicine, and Third (NYU) Medical and Surgical Divisions, Bellevue Hospital, New York City.

Tillett, Johnson, and McCarty(1) have described the effects produced by the intravenous infusion of streptokinase (SK) in man. They demonstrated that activation of the blood protease system could be produced by this means, and suggested that this phenomenon might be applied to the therapy of human thrombo-embolic disease. The available streptokinase preparations (Varidase®† assaying at 95 SK units/gamma N) not infrequently evoked reactions of the non-specific protein type consisting of pyrexia, hypotension, and malaise. However, it was demonstrated that premedication of the patients with antipyretic and antihistaminic compounds suppressed the reactions sufficiently to permit infusions of SK in amounts required to create intravascular fibrinolysis. Even though all commercial preparations of Varidase®, for therapeutic use, are devoid of pyrogenicity in rabbits, this type of animal testing has not been a uniformly satisfactory guide to the effect of the preparations when injected intravenously into patients. Previous preparations of streptokinase (contained in Varidase®) were known to be impure, and a study of purification methods has been an integral part of work designed to facilitate further evaluation of induced systemic fibrinolysis in man. It was important to determine, therefore, whether the reactions produced in man by Varidase® were due to impurities in the preparation or to the effects of streptokinase itself.

In the preceding article several methods of purification have been described(2). The purpose of the present investigation has been to test these more highly purified streptokinase preparations for their clinical effect in man and to indicate their potential advantage for practical therapy. Furthermore, it was desired to induce systemic fibrinolysis in man, without the use of the reaction suppressing drugs previously employed in order to observe the action of the SK activated protease without other conditioning factors.

Materials. Streptokinase purified by four different procedures was available for test (2). *Method 1 material* (assaying at 340-450 units/gamma N) was prepared by chemical fractionation followed by starch zone electrophoresis. *Method 2 material* (assaying at 390-430 units/gamma N) was prepared by a more complex fractionation procedure. *Method 3 material* (assaying at 410 units/gamma N) was method 2 material subjected to one further fractionation step. *Method 4 material* (assaying at 600 units/gamma N) was method 2 material subjected to starch zone electrophoresis.

Methods. Patients suffering from either a local inflammatory lesion, in some cases secondary to arterial or venous insufficiency, or from thrombophlebitis were selected for treatment. The clinical results of the therapy appeared in many instances to be favorable but will be described in a separate communication. The test material was mixed with 500 ml of 1% human albumin-physiological saline solution, and infused at a constant rate over a period of 4 hours. The patient's blood pressure, temperature, pulse and respiratory rates were recorded at hourly intervals, and electrocardiograms were taken, before, during and after treatment on patient's receiving 30,000 or more units of streptokinase. No drugs were administered during the infusion or observation period. The presence or absence of fibrinolysis in the peripheral blood was determined by previously described methods(1).

Results. The present studies made with the different purified preparations have been

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† Streptokinase-Streptodornase, Varidase®, Lederle Laboratories, Pearl River, N. Y.

TABLE I. Effect of Varying Doses of Different Lots of Purified Streptokinase in Man (128 Infusions in 61 Patients). Figure in front of slash in each column on the right shows No. of infusions at each dose level, and figure after slash shows No. of infusions that caused a clinical reaction. The figure in parentheses indicates No. of reactions that were of moderate severity (see text for criteria).

Method of preparation				Dose in SK units		
Method	Lots	Units/γ N	No. of patients	10,000-30,000	31,000-70,000	71,000-130,000
1	4	340–450	21	16/ 4	4/6	2/2 (1)
2	2	390–430	9	17/10 (2)	—	—
3	1	410	16	30/ 8	5/4 (1)	5/2 (2)
4	2	600	15	28/ 6	3/0	8/3 (3)

compared with the effects produced by intravenously injected Varidase®. In the previous report(1) on intravenous Varidase®, the patients received relatively large doses (80,000 to 150,000 units) and the febrile and hypotensive effects of the infused material were suppressed by the appropriate administration of amidopyrine and chlor-trimeton.

In addition, extensive previous experience with intravenous Varidase® (not reported in detail) without the use of suppression drugs and in wide ranges of dosage has shown that 2 out of 3 patients experienced a 2-3°F rise in body temperature, and 1 out of 4 patients experienced a fall of blood pressure following the infusion of 5,000-10,000 units of streptokinase. A dose of 10,000-20,000 units produced some pyrexia and hypotension in nearly all patients, together with malaise. A rigor was sometimes produced, while malaise, nausea and vomiting were occasionally noted in the absence of pyrexia. All symptoms subsided within 12 to 24 hours.

I. *Comparison of effects of preparations made by each of 4 methods.* Three grades of reaction to the test infusion were recognized. The patients were assumed to have had no reaction, if no symptoms or signs were detected following the infusion. A *mild reaction* was diagnosed if the temperature was raised 1°F, if the systolic blood pressure fell 20 mm of mercury or the diastolic blood pressure fell 10 mm mercury, or there were mild untoward symptoms. A greater rise of body temperature (more than 1°F), a greater fall of blood pressure, or untoward symptoms other than mild were regarded as indicative of a *moderate reaction.* Patients who experienced moderate reactions were usually treated with antipyretics, or sometimes with both antipyretics and antihistaminics after termination of the infusion. Such therapy produced rapid symptomatic relief with a prompt fall of body temperature to normal limits. Patients tended to react less to a second injection of streptokinase than to a first injection. This source of error does not invalidate the comparison between the various batches of material tested as the phenomenon was inconstant and approximately equal numbers of first and second infusions were made in each group.

The results with 4 different lots of SK, purified by different methods, have been tabulated at 3 dose ranges: low, 10,000 to 30,000 units; moderate, 31,000 to 70,000 units; high, 71,000 to 130,000 units. These ranges were selected so that the majority of patients in each group received approximately the mean dosage in each range. The infusion data for the various lots are summarized in Table I.

Table I shows that the toxicity of material prepared by method 2 was greater than that prepared by the other 3 methods. The data are insufficient to decide between the merits of the other 3 methods though they suggest that method 4 was the best. The incidence and degree of pyrexia, hypotension and untoward symptoms experienced by patients who received 100,000 units of material prepared by methods 1, 3 and 4, was closely similar to that experienced by patients who received 5,000 units of Varidase®. For this reason, it was concluded that the purification of streptokinase, in the manner described, had reduced its toxicity to man by approximately 95%.

TABLE II. Clinical Effect of Systemic Fibrinolysis. Following infusion of comparable amounts of streptokinase, one group of patients (lysis group) developed an active lytic system in the blood, while the other did not (control group). Clinical response of these groups is shown in table (for criteria of reactions see text).

Clinical effect	Control group (no lysis) No. of infusions		Lysis group No. of infusions	
No reaction	12 (35%)	25 (73%)	4 (27%)	13 (87%)
Mild "	13 (38%)		9 (60%)	
Moderate reaction	9 (27%)		2 (13%)	
Total No. of infusions	34		15	
No. of patients	26		12	

Caution was observed in the clinical trials of each of the preparations and as a consequence, the majority of patients received insufficient quantities of streptokinase to activate their blood protease systems. However, high levels of fibrinolytic enzyme were detected in the blood of 15 patients following the streptokinase infusion. Blood, withdrawn from the patients at the end of the infusion and for some hours afterwards, showed spontaneous clot lysis in 45-240 minutes(1). The symptoms and signs shown by these patients who received streptokinase and exhibited fibrinolytic activity in the blood stream, were compared to a similar group, who received the same dose of streptokinase but who failed to show clot lysis (Table II). Though the number of observations is small, it appears that the two groups experienced a similar incidence and degree of clinical reaction. The fact that 13 out of 15, or 87%, of the infusions in the patients with lysis were followed by no more than a 1°F rise in temperature, or a small fall of blood pressure suggests that high levels of circulating fibrinolytic activity are tolerated in man without significant symptomatology.

Electrocardiographic studies. Since intravenous infusions of SK will be employed as specific treatment in selected patients with cardiovascular disease, a number of patients who received infusions of streptokinase, prepared by these and other methods, were followed with extensive electrocardiograms. Kellner and Robertson(3) recently demonstrated that injection of large doses of streptokinase into rabbits caused cardiac damage. Neither clinical nor electrocardiographic examination of these patients have shown evidence of such an effect in man. 165 electrocardiograms were taken on 60 patients, most of whom were in the older age groups. Standard limb leads, unipolar extremity and precordial leads were recorded by means of a Cambridge string galvanometer. An electrocardiogram taken prior to infusion of streptokinase acted as control for subsequent tracings taken on each patient during, immediately after, or within 12 hours following the infusions.‡

No abnormality was evident before or after the infusions in 114 electrocardiograms taken on 47 of these patients free of heart disease.

It seemed particularly significant that there was no change in 31 abnormal electrocardiograms taken before, during and after the SK infusions on 9 patients who had heart disease. This group included 2 patients with severe hypertensive and arteriosclerotic heart disease and 5 with moderately severe arteriosclerotic heart disease complicated by recurrent congestive heart failure and/or cardiac arrythmias. The electrocardiogram of another patient showed premature ventricular systoles and inverted T waves in leads I, II, AVL, V3 and V5 with no other evidence of cardiac disease. The last patient in this group, with moderately severe heart disease

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of unknown origin, had congestive heart failure on three different occasions and was on a maintenance dose of digitalis. The average age of these patients was 67 years.

The electrocardiograms of 4 other patients who had abnormal T-waves in their control tracings showed minor changes in these abnormalities during the period of observation and infusion. One of these patients had no previous evidence of heart disease except an electrocardiographic abnormality, one had hypertensive and arteriosclerotic heart disease and 2 had arteriosclerotic heart disease. The tracings on one of the patients with arteriosclerotic heart disease had deeply inverted T waves in V3 before the infusions which became less inverted during and after the infusions, while those on the patient with hypertensive and arteriosclerotic heart disease had low T waves in nearly all leads which also became more positive during the infusion period. Similarly, the noncardiac patient had an inverted T in lead AVL in his control tracing which became more positive as the infusions progressed. The last patient was found to have frequent premature ventricular contractions prior to, and following the infusions and a short run of ventricular tachycardia on one of the 9 infusion days. These changes were not associated with any symptoms, the amount of SK administered or with simultaneous laboratory studies including the serum oxaloacetic transaminase.

In short, the few changes that did occur in these electrocardiograms were minor and inconsistent and therefore could not be ascribed to the effect of the infusion.

Discussion. The clinical trial, during which 128 infusions of streptokinase were administered to 61 patients, shows that the toxicity of streptokinase to man can be greatly reduced by purification. It appears that methods 1, 3 and 4 produce material that is only 1/20th as toxic to man as is Varidase®. The toxicity comparison between methods 1 and 2, both producing material of almost identical potency but of differing clinical toxicity, suggests that toxicity may be unrelated to the administration of streptokinase as such, but rather due to the presence of a contaminating substance. Immunochemical analysis has shown that all the present preparations contained more than one antigenic component, though a positive identification of these antigens has yet to be made.

The observation that high levels of circulating fibrinolytic enzyme produce no symptoms in man is of theoretical interest as well as of practical importance. It has been repeatedly suggested (Reviews 4, 5) that activation of the blood protease system, leading to the liberation of biological split products (anaphylatoxins) was the cause of, or played an important role in the production of the hypersensitivity reaction. The demonstration that histamine release and blood protease activation occurred during this state after union of circulating antigen with sensitized tissue(6,7) that antigen-antibody union may activate serum protease *in vitro*(8), and that in some instances, at least, proteolysis and histamine release were correlated in time and amount(9) have maintained interest in this mechanism. Our findings, that activation of the blood protease system in man is not accompanied by clinical symptomatology, seem to exclude participation of the plasminogen-plasmin system in the pathogenesis of the hypersensitivity reaction.

Summary. 1. The clinical effects of specially purified streptokinase preparations have been determined in man. Streptokinase, 6-7 fold purer than that previously available, has been shown to have a greatly diminished toxicity, when administered by the intravenous route. The best of these preparations have only 1/20th of the toxicity of the previous preparations. 2. Systemic fibrinolysis has been induced in man by infusion of these preparations, unprotected by reaction suppressing drugs. A comparison between those patients who showed peripheral blood fibrinolysis, and those who did not, indicated that this state is asymptomatic in the human. 3. 165 electrocardiograms taken on 60 patients, before, during and after the infusion of streptokinase showed few changes and these were of minor degree. Since they followed no consistent pattern it was not pos-

sible to ascribe the changes to the effect of the infusion.

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Cardiotonic Activity of 9-Alpha Fluorohydrocortisone.* (22914)

RALPH D. TANZ,† RICHARD W. WHITEHEAD, AND G. J. WEIR, JR.
Department of Pharmacology, University of Colorado School of Medicine, Denver.

Previous work from this laboratory(1) reported the positive inotropic action of 9-alpha fluorohydrocortisone‡ (9-a FF), and its effect upon Na^{22} distribution in the papillary muscle preparation. In the present paper evidence is presented showing the positive inotropic action of 9-a FF in low concentrations, and its negative inotropic action in higher concentrations. These findings are correlated with histological changes in cardiac muscle and intramuscular Na^{22} and K^{42} movement.

Methods. 1. *The cat papillary muscle preparation* first described by Cattell and Gold(2) was employed with certain modifications(1). Briefly, 2 muscles were isolated from the right ventricle without causing gross injury. A control and a treated muscle were stimulated in two identical chambers at 60 times per minute throughout the experiment. Krebs-Ringer bicarbonate solution enriched with dextrose was used as the bathing fluid maintained at 38°C and a mixture of 95% O_2 and 5% CO_2 bubbled through it. A minute amount of either Na^{22} or K^{42}§ was added to the bathing fluid, the amount of K^{42} added being taken into account when preparing the medium. 9-a FF in amounts indicated in Table I was then added to the bathing fluid of one of the muscles. The uptake of isotopic Na or K was expressed in counts/sec/mg and then in terms of the ratio of radioactivity of the samples treated with 9-a FF to that of control samples. 2. *The isolated heart preparation* as modified by Anderson and Craver (3) was employed using whole cat hearts and enriched Krebs-Ringer bicarbonate solution as the perfusing medium. Failure was induced by perfusing the preparation with fresh, unused perfusate(4). Since 9-a FF in a concentration of 0.5 μg/ml caused a positive inotropic action in the papillary muscle preparation, this concentration was added to the reservoir bottle and perfused through the heart after signs of cardiac failure appeared. 9-a FF 'toxicity' was induced by adding 10 μg/ml to the perfusate at 0 time, as indicated in Fig. 2. 3. *Histologic sections* were obtained from fresh papillary muscles or those stimulated at 60 times per minute for 6 hours in concentrations of 0, 0.5 or 20 μg/ml

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† U.S.P.H.S. Fellow of N.H.I.

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§ Specific activity of Na^{22} = 0.75 μc/ml (and amounted to 0.48 mEq/l in 143 mEq/l of Na); specific activity to K^{42} = 0.047 μc/ml (and amounted to 0.4 mEq/l in 5.66 mEq/l of K).