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Plasma Deoxyribonuclease and the Quantitative Effects of Injected DNA.* (26773)

J. PHILIP SAVITSKY† (Introduced by Bernard A. Sachs)

Lucy and Henry Moses Research Laboratories, Medical Division, Montefiore Hospital, N. Y.

It has been reported from this laboratory that a single injection of purified, undenatured deoxyribonucleic acid (DNA) produced in rabbits a significant decrease in serum cholesterol lasting at least 2 years(1,2,3). DNA preparations from homologous and heterologous mammalian tissues were both effective. The lowered mean serum cholesterol largely reflected the decline in cholesterol levels of those animals with high normal values. Quantitative studies of varying the amount of DNA injected over a 50-fold range showed a significant dose-response relationship(3).

One problem which arises in interpreting the quantitative data is that these animals have a circulating, potent plasma deoxyribonuclease. Some of the injected DNA molecules must be inactivated, denatured, or even degraded prior to their absorption by the cells of the organism. The enzyme circulating in the blood is similar to the pancreatic deoxyribonuclease I in its properties and requirements for activity(4). Mg^{++} ion is an essential activator and citrate is an effective inhibitor which acts by binding the Mg^{++}(5). The present study describes the inhibition *in vivo* of the plasma deoxyribonuclease by sodium citrate and the effect of this inhibition on the physiologic activity of injected DNA.

Methods. DNA used for injection was prepared from fresh beef liver by the mild procedures previously described(1), *i.e.*, extraction with high ionic strength salt and deproteinization with chloroform. The N/P ratio was 1.75. The fibres were dissolved in either isotonic saline or .015M sodium citrate plus isotonic saline, and injected immediately. The DNA used as the substrate for the enzyme determinations was prepared in identical fashion and dissolved in isotonic saline to a concentration of 0.15%. This was heated to 60°C for 30 minutes to destroy any residual deoxyribonuclease, placed in 10 ml aliquots and stored frozen at –15°C. The relative viscosity was 4.2.

The bloods were drawn into heparin in an ice bath, then centrifuged at 3000 r.p.m. for 30′ in a refrigerated centrifuge at 2°C. The supernatant plasma, apparently free of formed elements, was removed and stored frozen at –15°C. Deoxyribonuclease activity was determined by adding 1 ml of the heparinized rabbit plasma and 0.1 ml .02M $MgCl_2$ to 2 ml of the DNA substrate solution in an Ostwald viscosimeter at 37°C. The run-through time of the viscosimeters was approximately 20 seconds. Following a mixing period of one minute, viscosity measurements were made at 3 minute intervals for 30 minutes. Under these conditions, the straight-line portion of the curve lasted 18 minutes.

Total serum cholesterol was determined by a Bloor(6) procedure with 10 ml of the Bloor's reagent containing the extracted serum being taken to dryness.

Albino rabbits of the New Zealand strain weighing 2-3 kg were kept in temperature-controlled, air-conditioned, separate quarters and fed a standard chow *ad libitum*. All the

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† Established Investigator, American Heart Assn.

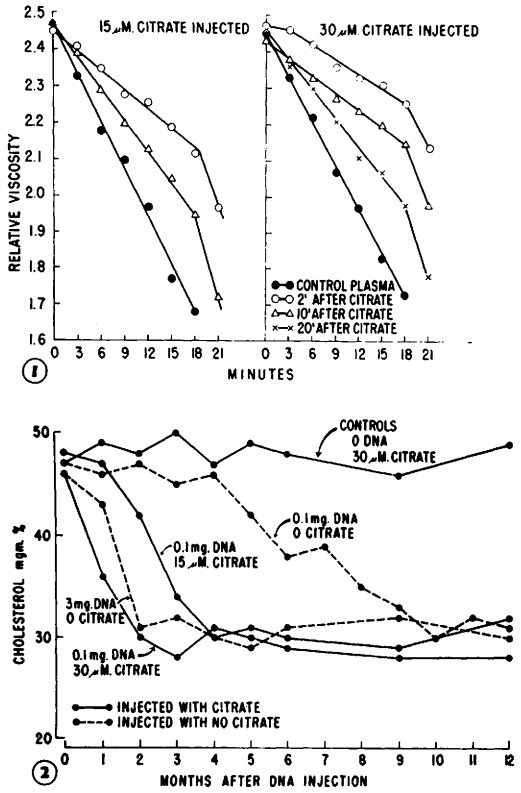

FIG. 1. Altered plasma deoxyribonuclease activity following sodium citrate inj.

FIG. 2. Avg serum cholesterol levels following inj. of DNA, with and without sodium citrate.

animals were kept at least 6 weeks in these quarters, prior to use, to permit them to adapt to the new, constant environment. Only those rabbits were used which ate well, showed a steady gain in weight and no evidence of disease.

Results. Sodium citrate solution (.015M $Na_3C_6H_5O_7$ + .15M NaCl) was rapidly injected intravenously into rabbits in amounts ranging from 9 μMoles to 150 μMoles. The animals were bled from the ear vein into heparin prior to injection and at 2′, 10′, 20′ and 30′ intervals after the citrate injection. The plasma samples were tested for their deoxyribonuclease activity as indicated above. A transient, but significant, inhibition of plasma deoxyribonuclease was observed in all the animals injected with the citrate solution. Fig. 1 demonstrates typical changes in 2 rabbits; one injected with 15 μMoles and one with 30 μMoles of citrate. In the animal receiving 15 μMoles there is a deoxyribonuclease inhibition of about 40%, 2′ after citrate injection and a gradual return to control levels within 20′. The plasma of the rabbit injected with 30 μMoles showed 60% inhibition and 30′ were required to return to the control activity. Injection of greater amounts of citrate resulted in the same magnitude of enzyme inhibition (60%) with the effect lasting longer.

The data indicated that *in vivo* plasma deoxyribonuclease activity was inhibited by injection of sodium citrate which, presumably, removed some of the necessary Mg^{++} ions. The question arose as to whether these small quantities of citrate, when diluted hundredfold into the total plasma volume, were capable of binding sufficient Mg^{++} to produce the observed inhibition. To check this, studies were done in which an equal volume of .015M $MgCl_2$ was injected separately into another vein immediately after the .015M sodium citrate injection. The injection of the small quantity of Mg^{++} completely prevented the citrate-induced enzyme inhibition.

The effect of plasma deoxyribonuclease inhibition on the quantitative physiologic activity of injected DNA was then determined. The same preparation of heterologous DNA was injected intravenously into several groups of rabbits, with and without sodium citrate, and the effects on serum cholesterol levels were studied. The animals were divided into 5 experimental groups with 10 normal, healthy rabbits in each group receiving the following injection: 1) controls—30 μMoles of sodium citrate in isotonic saline, 2) 0.1 mg DNA in saline, 3) 3 mg DNA in saline, 4) 0.1 mg DNA plus 15 μMoles sodium citrate in saline, 5) 0.1 mg DNA plus 30 μMoles sodium citrate in saline. The DNA injections did not produce any toxic effects, acute or chronic. There was no difference in nutrition, weight gain or general health between injected and control groups. The animals were bled from the ear vein prior to injection and at monthly intervals thereafter for 12 months. Total serum cholesterol was measured on all the bloods.

Fig. 2 demonstrates the changes in the average serum cholesterol level of the 5

groups of rabbits over a period of one year after the injections. The altered serum cholesterol appeared more rapidly in those animals receiving DNA and citrate. The changes observed in the group injected with 0.1 mg DNA and 30 μMoles citrate were comparable to those of the group injected with 30 times as much DNA without citrate. Previous quantitative studies(3) had shown that over this same dosage range the time required for the appearance of the serum cholesterol changes varied inversely with the amount of DNA injected. Therefore, it appeared that *in vivo* inhibition of the plasma deoxyribonuclease by sodium citrate in this experiment had increased 30-fold the effectiveness of the injected DNA.

Discussion. The data presented indicate that injection of sodium citrate produces a significant *in vivo* inhibition of the deoxyribonuclease circulating in blood. The effectiveness of the injected DNA in lowering serum cholesterol levels was markedly increased when it was injected together with citrate. Injection of 0.1 mg DNA with a measurable, transient, enzyme inhibition lasting 30 minutes resulted in quantitative effects comparable to 3 mg DNA injected without citrate. This 30-fold greater effect suggests that the DNA injected without citrate in this, and in previous studies(1,3), has been largely inactivated (at least 97%) in the bloodstream prior to absorption into cells. It also serves to emphasize the exceedingly small quantities of "active" DNA needed to induce the prolonged alterations of serum cholesterol regulation.

It is noteworthy that a 2-fold inhibition of the plasma deoxyribonuclease as determined by viscosity measurements was associated with a 30-fold increase in physiologic activity of the DNA. A similar disproportion between viscosity changes and biologic activity was observed by Zamenhof and his colleagues (7) in the effect of crystalline deoxyribonuclease on bacterial transforming DNA. Incubation of small amounts of the enzyme with the DNA resulted in loss of 100% of the biologic activity and only a 10% decrease in the viscosity.

Summary. Sodium citrate injected into rabbits in amounts ranging from 9 μMoles to 150 μMoles produces a transient, significant *in vivo* inhibition of the plasma deoxyribonuclease. Injection of heterologous DNA into rabbits induces a prolonged decrease in total serum cholesterol. 30 μMoles of citrate and 0.1 mg DNA, injected together, are quantitatively as effective as 3 mg DNA in altering the serum cholesterol regulation. The citrate-induced deoxyribonuclease inhibition increased the physiologic activity of the injected DNA as much as 30-fold.

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In vivo P^{32} Radioautography in Detecting Breast Cancer.* (26774)

ARTHUR S. MCFEE, NORMAN B. ACKERMAN, AND OWEN H. WANGENSTEEN
Department of Surgery, University of Minnesota Medical School, Minneapolis

A method of *in vivo* radioautography of the stomach based on selective uptake of P^{32} by malignant tissue, and employing a balloon coated with a photosensitive emulsion, has been described(1,2). Adaptations of this method for various other organs are being explored. The purpose of this communication is to report upon our experience with

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