**Table I—Serum Protein Binding Parameters for Prednisolone in Four Species Obtained by Methods I and II [a]**

| Species | Transcortin | | | | Albumin | | |
|---|---|---|---|---|---|---|---|
| | $P_T, M \times 10^7$ | | $K_T, M^{-1} \times 10^{-7}$ | | $P_A$ [b], $M \times 10^4$ | $K_A, M^{-1} \times 10^{-3}$ | |
| | I | II | I | II | | I | II |
| Rabbit | 5.74 | 6.13 | 2.33 | 2.09 | 4.49 | 6.22 | 6.15 |
| Dog | 2.21 | 1.19 | 0.284 | 0.569 | 5.73 | 2.62 | 2.69 |
| Rat | 0.259 | 1.01 | 0.653 | 0.152 | 3.96 | 1.51 | 1.45 |
| Human | 6.19 | 3.26 | 0.499 | 1.63 | 6.43 | 1.21 | 1.400 |

[a] Method I is from Ref. 4, and Method II is from Ref. 5. [b] Determined by an automatic analyzer.

trations are of less significance, accounting for the good agreement in the $K_A$ values between the two methods.

The binding profile of the rabbit appears most like humans in the degree of nonlinearity in prednisolone binding (Fig. 1). The binding constants in Table I (Method II) confirm this observation. The $P_T$ and $K_T$ values in the rabbit are closest to those values determined in humans, producing similar decreases in binding as the serum prednisolone concentration increases. The binding parameters confirm the presence of high affinity, low capacity transcortin binding and low affinity, high capacity albumin binding in each species.

The transcortin concentrations and affinity constants for all of the species examined are in the general range of values reported by other investigators for cortisol. Westphal (6) obtained $P_T$ and $K_T$ values in the rabbit of $3.6 \times 10^{-7}$ $M$ and $4 \times 10^7$ $M^{-1}$, which agree well with the present estimates. In contrast, the $P_T$ and $K_T$ values determined by Westphal in the rat were $6.9 \times 10^{-7}$ $M$ and $1 \times 10^7$ $M^{-1}$. These discrepancies may reflect inherent differences in the interactions of prednisolone and hydrocortisone with transcortin. The use of different strains of the same animal species and physiological factors such as age, sex, and endogenous corticosteroid level might affect the transcortin–steroid interaction and contribute to variability in the binding parameters (7).

In summary, the animal species studied differed markedly in their ability to bind prednisolone. The rabbit was the most similar to humans in transcortin concentration and affinity constant for prednisolone and in the range of free drug fractions at increasing steroid concentrations.

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## ACKNOWLEDGMENTS


Supported in part by Grant 24211 from the National Institutes of General Medical Sciences.

The MACMOL computer program (5) was kindly provided by Dr. Roger L. Priore.


# Effect of Sodium Bicarbonate on *In Vitro* Conversion of Fibrinogen to Fibrin


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Received November 8, 1979, from the *Division of Nuclear Medicine, Department of Radiology, Martin Luther King Jr. General Hospital and Charles R. Drew Postgraduate Medical School, Los Angeles, CA 90059.* Accepted for publication March 12, 1980.



**Abstract** □ The effect of sodium bicarbonate on the conversion of fibrinogen to fibrin clot was investigated using fresh human whole blood and pure human fibrinogen. *In vitro* experimental data demonstrated that sodium bicarbonate interfered with fibrin clot formation during clotting. This study assessed the possibility of synergistic action of sodium bicarbonate with sodium citrate. As expected, sodium citrate potentiated the anticoagulant action of sodium bicarbonate.



**Keyphrases** □ Sodium bicarbonate—effect on conversion of fibrinogen to fibrin, synergistic action with sodium citrate □ Sodium citrate—effect on fibrin clot formation, synergistic action with sodium bicarbonate □ Fibrin—effect of sodium bicarbonate and sodium citrate on *in vitro* conversion of fibrinogen


*In vitro* experimental results and clinical patient data demonstrate that sodium and potassium bicarbonate can interfere with the clotting process, as evidenced by prolongation in prothrombin time and thrombin clotting time (1). The anticoagulant effect appears to be caused primarily by the specific anion species and an increase in ionic strength and to a lesser extent by the effect of pH. Thrombin clotting time analyses on citrated whole blood samples containing sodium bicarbonate indicate that the unavailability of calcium ions is not the direct cause of clot inhibition.

Since previous work contained no information dealing directly with the effect of bicarbonate on clotting (2–9), the present study was undertaken to determine the effect of sodium bicarbonate on the conversion of pure human fibrinogen to fibrin clot. The possibility of synergistic action of sodium bicarbonate with sodium citrate on clot inhibition also was investigated.

## EXPERIMENTAL

Lyophilized human fibrinogen[1] was reconstituted with water for injection USP to a concentration of 20 mg/ml at pH 6. Prior to the addition of fibrinogen, a predetermined amount of sodium bicarbonate solution[2] (pH 7.8, 7.5%), ranging from 1 to 25 mg in a volume of <0.1 ml, was added to each test tube containing 3.0 ml of 7 m$M$ pH 7.4 Sorensen's phosphate buffer (ionic strength 0.017) and 0.4 ml of a 3.8% sodium citrate solution (pH 9.85, 0.13 $M$, ionic strength 0.078). The resulting mixture contained one part of citrate solution to nine parts of test sample. The amount of citrate solution used was equivalent to the quantity of anticoagulant present in the blood-collecting test tube. When the required amount of sodium bicarbonate solution to be added to the test samples exceeded 0.1 ml, a weighed amount of reagent grade powder[3] was dissolved in the

[1] Parenogen, Cutter Laboratories, Berkeley, Calif.
[2] Abbott Laboratories, North Chicago, Ill.
[3] American Drug and Chemical Corp., Los Angeles, Calif.




*0022-3549/80/0800-0978$01.00/0*

Table I—*In Vitro* Anticoagulant Action of Sodium Bicarbonate on Fresh Human Blood Samples

| Sample | Sodium Bicarbonate Concentration[a] mEq/kg of Body Weight | mEq/ml of Blood | Total $\mu$ | pH | Prothrombin Time, sec | Thrombin Clotting Time, sec |
|---|---|---|---|---|---|---|
| Control | 0.00 | 0.0000 | 0.154 | 7.40 | 11.1 | 7.5 |
| 1 | 0.81 | 0.0125 | 0.167 | 7.40 | 11.3 | 8.1 |
| 2 | 1.62 | 0.0250 | 0.179 | 7.40 | 11.8 | 9.0 |
| 3 | 2.43 | 0.0375 | 0.192 | 7.50 | 12.3 | 9.5 |
| 4 | 3.24 | 0.0500 | 0.204 | 7.65 | 13.3 | 10.5 |
| 5 | 4.05 | 0.0625 | 0.217 | 7.65 | 14.1 | 12.1 |
| 6 | 4.86 | 0.0750 | 0.229 | 7.65 | 15.3 | 14.0 |
| 7 | 5.67 | 0.0875 | 0.242 | 7.65 | 17.0 | 16.5 |
| 8 | 6.48 | 0.1000 | 0.254 | 7.65 | 19.8 | 18.0 |
| 9 | 7.29 | 0.1125 | 0.267 | 7.70 | 22.5 | 21.0 |
| 10 | 8.10 | 0.1250 | 0.279 | 7.74 | 26.0 | 32.0 |

[a] The amount of sodium bicarbonate added to the samples ranged from 12.5 to 125 mEq/liter of blood, assuming an average adult blood volume of 65 ml/kg of body weight. The plasma ionic strength, $\mu$, was 0.154. All values represent the average of three determinations from three normal healthy volunteers.

sodium citrate–phosphate buffer mixture. Exactly 0.6 ml of fibrinogen solution (12 mg of fibrinogen) was added to each test tube, yielding a final concentration of 3 mg of fibrinogen/ml of sample. (Normal blood fibrinogen concentration ranged from 150 to 350 mg %.) Similar samples but without sodium citrate were prepared to eliminate any synergistic effect of sodium citrate with sodium bicarbonate.

After thorough mixing, the pH of each sample was determined at 37° by a pH meter[4] with a combination glass and ceramic electrode. Coagulation was induced by the addition of 0.1 ml (10 units/ml, pH 6) of topical thrombin[5] in normal saline to 0.2 ml of the test sample previously incubated at 37° for 2–5 min. The thrombin clotting time (expressed in seconds) was recorded mechanically with an automatic clot timer[6]. Control samples were assessed without sodium bicarbonate. The ionic strength of the phosphate-buffered fibrinogen solution in the absence of sodium bicarbonate and sodium citrate was 0.017. With the addition of sodium citrate, the total ionic strength was 0.095.

To determine the effect of sodium bicarbonate on clotting, *in vitro* studies were performed using fresh blood samples collected from healthy volunteers by venipuncture. Prior to addition of the whole blood, a predetermined amount of sodium bicarbonate solution or powder was added to specimen test tubes used for blood collection, each containing 0.3 ml of 3.8%, 0.13 *M* sodium citrate. Exactly 2.7 ml of the fresh whole blood was added immediately to the sample test tube and mixed well. The quantity of sodium bicarbonate added to the blood samples was equivalent to administration of 1–10 mEq/kg to a 70-kg adult, assuming uniform distribution only in the blood.

The blood sample pH was determined prior to plasma separation. Plasma was separated from the whole elements by centrifugation at 2000 rpm (540×*g*) for 30 min. One-stage prothrombin time, by the addition of thromboplastin and calcium ions to 0.2 ml of plasma, was determined according to standard laboratory methods. The same plasma sample was diluted to 1:10 with Owren's pH 7.4 barbital[7] (barbital sodium–sodium chloride) buffer. Thrombin clotting time was determined by adding 0.1 ml (1 unit) of topical thrombin to 0.1 ml of a plasma sample previously incubated at 37°. Both the prothrombin time and the thrombin clotting time were recorded with an automatic clot timer. The plasma ionic strength was assumed to equal that of normal saline (0.9% NaCl), which is 0.154. Control samples were tested without the addition of sodium bicarbonate.

## RESULTS

The inhibitory effect of sodium bicarbonate on the conversion of fibrinogen to fibrin clot is illustrated in Fig. 1. At the salt concentration used, the ionic strength of the bicarbonate samples increased from a control value of 0.017 to 0.317 and that of the sodium citrate–sodium bicarbonate samples rose from a control value of 0.095 to 0.395. Thrombin clotting time increased gradually from 6.9 to 8.6 sec as the bicarbonate concentration increased from 0.012 to 0.12 mEq/ml (1–10 mg/ml). The thrombin clotting time of the control sample was 6.4 sec. Increasing the bicarbonate content beyond 0.12 mEq/ml caused a sharp rise in the thrombin clotting time from 10 to 156 sec.

As seen in Fig. 1, sodium citrate potentiated the anticoagulant effect of sodium bicarbonate. While the sodium citrate concentration remained constant at 3.8 mg/ml, the addition of sodium bicarbonate to these samples markedly prolonged the thrombin clotting time.

The pH of the bicarbonate samples gradually rose from 7.2 to 7.8 with the increasing amount of salt added. A similar increase in pH, from 7.2 to 7.7, was observed with sodium citrate–sodium bicarbonate samples. However, results from the pH determination did not demonstrate a linear relationship between the rise in pH and the sharp increase in the thrombin clotting time.

Table I summarizes the *in vitro* prothrombin time and thrombin

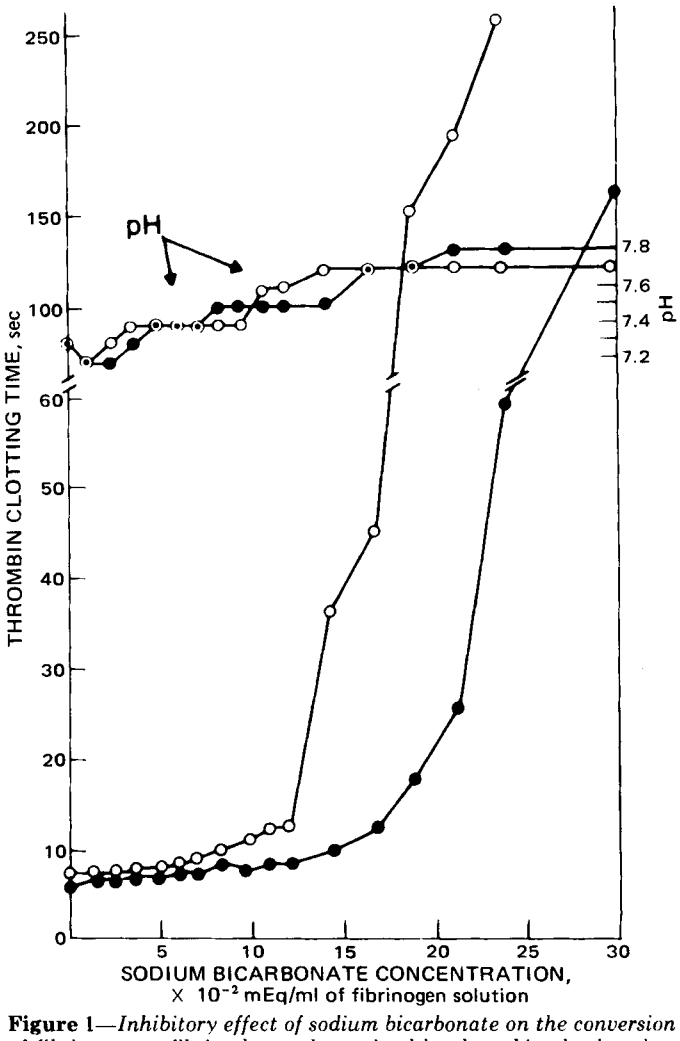


**Figure 1**—*Inhibitory effect of sodium bicarbonate on the conversion of fibrinogen to fibrin clot as determined by thrombin clotting time assay. Sodium citrate potentiated the anticoagulant action of sodium bicarbonate. Key:* O, *sodium bicarbonate and sodium citrate; and* ●, *sodium bicarbonate alone.*

[4] Instrumentation Laboratory, Lexington, Mass.
[5] Parke-Davis, Detroit, Mich.
[6] Fibrometer, Becton-Dickinson, Rutherford, N.J.
[7] Veronal, Dade Division, American Hospital Supply Corp., Miami, Fla.

clotting time data on fresh blood samples containing various amounts of sodium bicarbonate. The amount of sodium bicarbonate added to the samples ranged from 12.5 to 125 mEq/liter of blood, assuming an average adult blood volume of 65 ml/kg. At added bicarbonate concentrations of 1–2 mEq/kg, significant changes in clotting were observed. Both the prothrombin time and the thrombin clotting time were elevated (Samples 1 and 2, Table I). Increasing the bicarbonate concentration beyond 2 mEq/kg inhibited clotting markedly.

## DISCUSSION

Sodium bicarbonate is used widely as a therapeutic agent for acid–base imbalance (10–12). The usual dose of sodium bicarbonate ranges from 1 to 2 mEq/kg and normally is administered intravenously as a 7.5% hypertonic solution or as a 1.5% isotonic solution. Sodium bicarbonate has been regarded as a safe and effective therapeutic agent and is relatively free of serious side effects. With the exception of overdose-induced seizures or tetany secondary to hypocalcemia and hyperalkalosis, no known adverse reactions have been reported (13, 14).

Results from the present investigation demonstrate that sodium bicarbonate inhibits the conversion of fibrinogen to fibrin. Although the mechanism of clot inhibition is unclear, it is well known that some neutral salts retard coagulation and that others accelerate the conversion of fibrinogen to fibrin (2–9). Both cations and anions may bind to the fibrinogen or fibrin molecules during clot formation, and, depending on the individual chemical species, they can either accelerate or inhibit coagulation. The degree of clot inhibition also is affected greatly by the concentration and ionic strength of the salt.

Although data from pH determinations do not correlate well with observed changes in the prothrombin time and the thrombin clotting time, the effect of increasing pH on clotting cannot be ignored. Previous studies revealed that the structure and properties of fibrin clots are modified greatly by variations in pH and ionic strength during coagulation. However, even at constant pH and ionic strength, certain ions and neutral molecules at low concentration greatly affect the structure of the fibrin clot and its formation rate.

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# Polydimethylsiloxane Pellets for Sustained Delivery of Morphine in Mice


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Received February 11, 1980, from the *Departments of Pharmacology and Pharmaceutics, Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.* Accepted for publication March 13, 1980.



**Abstract** □ A new dosage form was designed whereby a polymeric silicone elastomer pellet provided sustained delivery of morphine to mice over 11 days. These pellets, which can be made easily and inexpensively with a standard tablet mold, gradually released morphine sulfate into the implanted mice. Maximal morphine-induced physical dependence, measured by jumping during naloxone-induced withdrawal, was observed 3–5 days after implantation. At this time, slightly less than 50% of the morphine sulfate had been released. Drug release continued through Day 11 and was accompanied by a physical dependence of decreased magnitude compared to that observed on Day 3 or 5.



**Keyphrases** □ Morphine—sustained release from polydimethylsiloxane pellets, mice □ Drug delivery systems—sustained release of morphine from polydimethylsiloxane pellets, mice □ Sustained-release systems—delivery of morphine from polydimethylsiloxane pellets, mice


As a representative of the narcotic analgesic drugs, morphine has been used to induce tolerance and physical dependence in experimental animals (1). Its relatively short half-life in small animals requires frequent injections of morphine to sustain drug levels over an extended period.

## BACKGROUND

Attempts have been made to deliver morphine to experimental animals without these injection routines but with the production of maximum tolerance and dependence. For example, Collier *et al.* (2) used an oily sustained-release preparation. Hui and Roberts (3) adsorbed morphine sulfate onto molecular sieves. Goode (4) used an implanted silicone rubber tubing reservoir of morphine solution. Teiger (5) used continuous intraperitoneal infusion. The usefulness of several of these methods is marginal as judged by the lack of utilization by fellow researchers. According to this criterion, employment of implanted tableted pellets that slowly release their morphine content appears to be the most accepted method to date.

The most commonly used morphine implant is the microcrystalline cellulose pellet described by Gibson and Tingstad (6). However, this pellet has several disadvantages as a drug delivery system for morphine. One disadvantage is that drug delivery in the animal ceases after 3 days, with only half of the drug being released from the pellet for absorption; the pellet tends to become encapsulated by membranous tissue, thereby diminishing morphine bioavailability (7, 8). Another disadvantage is that the pellet quickly becomes soft and mushy, so complete removal of the implant from the animal is difficult. This result is due to the fact that the pellet contains ~50% microcrystalline cellulose, a tablet disintegrant. Failure to remove all of the implanted pellet gives erroneously high values




*0022-3549/80/0800-0980$01.00/0*