

Contraceptive Properties of *Stevia rebaudiana*

Abstract. *A water decoction of the plant Stevia rebaudiana Bertoni reduces fertility in adult female rats of proven fertility. The decoction continues to decrease fertility for at least 50 to 60 days after intake is stopped. The decoction did not affect appetite and apparently did not affect the health of adult rats.*

Stevia rebaudiana Bertoni (Compositae) is a Paraguayan weed which contains a surprisingly sweet principle called stevioside (1). It is prescribed by some Paraguayan physicians as a hypoglycemic drug (2), although the hypoglycemic effect has not been confirmed. Paraguayan Matto Grosso Indian tribes use *Stevia rebaudiana* as an oral contraceptive. Women daily drink a decoction in water from dry, powdered leaves and stems.

Virgin females and females of proven fertility (one litter) all belonging to the Dispert colony of albino rats were used to investigate the effect of a decoction of *Stevia rebaudiana* on fertility. This is a strain of rats derived from 28 years of inbreeding the descendants of one pair. At the start of the experiments the age of the rats ranged from 90 to 152 days and their average body weight was 250 g. In experiment 1 virgin females (age 90 to 100 days), were divided into two groups of 14 rats each. One group drank 10 ml of a 5-percent decoction daily and the other group served as the control. All

of the rats received the same commercial poultry feed, and were housed individually in similar cages. To determine if the animals were experiencing regular estrous cycles, vaginal smears were taken daily in all experiments for 1 week before treatment started. After 12 days, the 28 females were mated with males of proven fertility. The decoction was continued daily throughout the 6-day mating period, but it was not available to the male rats. After a recovery period of 50 to 60 days during which rats did not receive the decoction, females of the experimental group were mated again under normal conditions.

In experiment 2, 14 female rats (age 140 to 152 days) were allowed to bear one litter. A week after the litter was weaned the females received the same dose of decoction for the same period of time as in experiment 1. They were then mated at random with males of proven fertility under the same conditions as above. Each female rat served as its own control. After a recovery period of 50 to 60 days without decoction, females of the experimental group were again mated.

In experiment 3, 14 female rats (age 102 to 114 days) were allowed to mate with males of similar age under normal conditions. A week after the litter was weaned (28 days after birth), the females were started with the same treatment as in experiments 1 and 2. After drinking the decoction for 12 days, they were mated under the same conditions as in experiments 1 and 2 with the same male that fathered their first litter.

Because of the lack of information concerning the amount of decoction that the Indians take, the calculations were made on the basis of our own experience acquired in northern Argentina with different weeds used as medicinals by the Indians. The Indians make a decoction with dry weeds (about 15 g) in water (about 300 ml)—or approximately 5 percent in weight of dry weed. If we consider that adult human females have an average body weight of 60 kg and drink daily a cup of decoction, a female rat weighing 0.250 kg should drink 1.25 ml (5 ml/kg) daily. We raised the dosage to eight times that quantity, or 10 ml per individual rat per day. The decoction was prepared daily by boiling the dry, powdered weed in water for 10 minutes and filtering after cooling. The decoction was administered orally by replacing the water bottles

with small bottles containing 10 ml of the decoction. As fast as each rat finished the decoction (usually in about 20 minutes), the normal water bottle was replaced.

Data are presented in Table 1. Fertility percentages were calculated on the basis of the number of litters as compared to the total females in each group. Fertility was reduced 57 to 79 percent in female rats drinking the decoction as compared to rats drinking water. A reduction of 50 to 57 percent in fertility was still evident 50 to 60 days after intake of the decoction had ceased. In experiment 1, eleven of the young belonging to two different litters of the experimental group lost their tails between the 12th and the 15th day of life as if they suffered a dry gangrene without visible cause. This abnormality was not observed in subsequent experiments.

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Table 1. Fertility of female albino rats after drinking an aqueous extract of *Stevia rebaudiana*. Each group consisted of 14 animals. Those in experiments 2 and 3 were of proven fertility.

Group	Average age (days)	Pregnant rats (No.)	Total offspring (No.)	Fertility (%)
<i>Experiment 1</i>				
Control	96	11	65	78
Experimental	98	3	17	21
Recovery mating*	153	4	21†	28
<i>Experiment 2</i>				
Control	101	14	91	100
Experimental	146	4	22	28
Recovery mating*	196	6	32	43
<i>Experiment 3</i>				
Control	108	14	86	100
Experimental	148	3	21	21

* Of experimental group. † One pup died, age 3 days; no abnormalities were observed.

Activation of Hageman Factor by L-Homocystine

Abstract. *L-Homocystine activates Hageman factor, as demonstrated by its capacity to initiate clotting and to induce the evolution of plasma kinins. Perhaps, strategically located deposits of this amino acid are responsible for the unusual frequency of thrombosis in patients with homocystinuria.*

Homocystinuria is a rare metabolic disorder inherited as an autosomal recessive trait (1, 2) in which cystathione synthetase, an enzyme needed to convert homocysteine to cystathione, is lacking (3). As a result, homocysteine cannot be metabolized, and is excreted in the urine in the form of homocystine (4).

Table 1. Adsorption of Hageman factor-like activity onto L-homocystine. Platelet-deficient citrated plasma was prepared from the venous blood of patients with Hageman trait or PTA-deficiency. The plasma was adsorbed with aluminum hydroxide gel (one-tenth volume; 0.55 percent in terms of aluminum oxide), and the adsorbed plasma was heated at 56°C for 30 minutes and centrifuged. Each plasma sample (1 ml) was shaken for 10 minutes in polystyrene tubes containing 2.7 mg of L-homocystine. The homocystine was separated by centrifugation, washed three times with 2 ml of barbital-saline buffer, and then suspended in 1 ml of this buffer. The suspension was then mixed with 0.1 ml of plasma deficient in Hageman factor, 0.1 ml of 0.1 percent crude soybean phosphatides in 0.15M sodium chloride, and 0.1 ml of 0.025M calcium chloride solution in polystyrene tubes (10 by 75 mm). The results are averages of duplicate determinations of the partial thromboplastin clotting time, at 37°C.

Treatment	Clotting time (sec)
Buffer	1050
Homocystine	1140
Homocystine exposed to Hageman factor-deficient plasma	850
Homocystine exposed to PTA-deficient plasma	440

A common, and frequently lethal complication of homocystinuria is arterial or venous thrombosis (1, 2, 5, 6). The pathogenesis of this intravascular clotting is unknown, although intimal (6) and medial (1) vascular fibrotic changes have been described. McDonald and his associates (7) suggested that thrombosis might be related to abnormal platelet "stickiness," but Schimke (1) quoted unpublished studies of D. P. Jackson and N. R. Shulman in which no abnormality in blood clotting, platelet adhesiveness or platelet survival could be demonstrated in homocystinuria.

Homocystine is only sparingly soluble in water; a saturated solution contains less than 30 mg per 100 ml. The concentration of this amino acid in the plasma of individuals with homocystinuria is as high as 5.4 mg per 100 ml (8). Conceivably, under some conditions, the local concentration of homocystine in body water may temporarily be sufficiently high that small amounts may precipitate in the intimal lining of

blood vessels, and in this way provide a focus for the formation of thrombi. To test this possibility, the clot-promoting properties of L-homocystine were examined.

Crystals of L-homocystine, added to normal human whole blood to provide a concentration of $10^{-2}M$ (9), decreased the clotting time from 34 to 10 minutes, as shown in the following way. One milliliter of normal human blood (10) was added to each of six polystyrene tubes, three of which contained 2.7 mg each of L-homocystine. Each tube was tapped twice and incubated at 37°C. After 5 minutes, one tube of each set, with or without L-homocystine, was tilted once a minute until its contents had clotted; thereafter, the second tube was tilted once a minute, and when its blood had clotted, the third. The clotting time recorded in all experiments was that of the third tube.

L-Homocystine also accelerated the coagulation of recalcified normal human citrated plasma which had not previously been in contact with clot-

promoting surfaces. In this experiment, 0.2 ml of platelet-deficient citrated normal plasma was mixed with 0.1 ml of L-homocystine, suspended in barbital-saline buffer (0.25M barbital-sodium barbital, 0.125M sodium chloride, pH 7.5), and 0.1 ml of 0.05M calcium chloride. The average of duplicate determinations of the clotting time, at 37°C, was 400 seconds when the concentration of L-homocystine in the mixture was $2.5 \times 10^{-2}M$, 473 seconds when $1.0 \times 10^{-2}M$, 697 seconds when $1.0 \times 10^{-3}M$, 780 seconds when $1.0 \times 10^{-4}M$, and 845 seconds when no homocystine was added. Thus, under the conditions used, L-homocystine was clot promoting at a concentration in the mixture of $10^{-3}M$. These experiments demonstrate that L-homocystine accelerated clotting by an action upon a plasma component or components; they do not preclude an additional effect upon the blood cells.

Many insoluble substances accelerate clotting by activating Hageman factor (Factor XII), a clot-promoting agent in normal plasma but not in the plasma of individuals with Hageman trait, a hereditary disorder (11). That L-homocystine may have exerted its effect by activating Hageman factor was tested by exposing the amino acid to plasma deficient in this factor or in plasma thromboplastin antecedent (PTA, Factor XI); the plasmas were first adsorbed with aluminum hydroxide gel and heated at 56°C to deplete them of clotting factors other than Hageman factor or PTA. L-Homocystine which had been treated with heated, adsorbed PTA-deficient plasma containing Hageman factor, accelerated the clotting of plasma deficient in Hageman factor (Table 1). In contrast, L-homocystine crystals treated with heated, adsorbed Hageman factor-deficient plasma containing PTA were much less effective. This experiment implies that Hageman factor was adsorbed onto crystalline homocystine, and in this form could correct the defect in plasma deficient in Hageman factor.

The possibility exists that, in this experiment, a clot-promoting agent other than Hageman factor had been adsorbed onto the homocystine. Besides its role in blood clotting, Hageman factor can bring about the elaboration of vasoactive polypeptide kinins (12). The effect of homocystine on the formation of kinins was tested by incubating platelet-deficient plasma and the amino acid, at a concentration of $8 \times 10^{-3}M$, in the presence of *o*-phenan-

Table 2. Effect of L-homocystine upon the elaboration of kinin-like activity. Platelet-deficient citrated plasma (0.1 ml), prepared in silicone-coated apparatus, 0.1 ml of 0.01M *o*-phenanthroline in barbital-saline buffer, and 0.8 ml of barbital-saline buffer or a suspension of 0.01M L-homocystine in buffer were shaken for 10 minutes at room temperature (12 by 75-mm tubes). Portions were added to an 8 ml bath of de Jalon's solution in which was suspended a segment of uterus removed from a rat injected intraperitoneally 24 hours before with 10 µg of diethylstilbestrol (in olive oil) per 100 g of body weight. The contraction of the uterus was recorded isotonically with a linear motion transducer, supplied with a constant frequency exciting voltage by an exciter-demodulator. The height of the contraction was compared with that of bradykinin in 0.15M sodium chloride; the bradykinin was added to the bath in a volume of 0.1 ml.

Test substance	Contraction of rat uterus (mm)
Bradykinin, 10 ng	42
Bradykinin, 20 ng	67
Bradykinin, 30 ng	68
Normal plasma + buffer, 0.1 ml	0
Normal plasma + L-homocystine, 0.1 ml	62
Normal plasma + L-homocystine, 0.05 ml	52
Normal plasma × L-homocystine, 0.03 ml	19
Hageman factor-deficient plasma + buffer, 0.1 ml	0
Hageman factor-deficient plasma + L-homocystine, 0.1 ml	0

throlin, a substance which inhibits kininase, an enzyme which destroys kinins (13). Mixtures of L-homocystine in normal plasma induced contraction of the rat uterus, while suspensions of the amino acid in plasma deficient in Hageman factor were without this effect (Table 2). Presumably, L-homocystine brought about the elaboration of kinin-like activity through its action upon Hageman factor.

Thus, at concentrations as low as $10^{-3}M$, the upper limit of its solubility, L-homocystine activates Hageman factor. It joins the lengthening list of agents which can activate this clotting factor (14). Hence, the unusual propensity of patients with homocystinuria to undergo thrombosis may be related to the deposition of homocystine in the intima of blood vessels. The validity of this hypothesis awaits the demonstration of homocystine in the tissues of patients with this disorder.

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9. This was not the amount in solution, but that amount which, if it had dissolved, would have provided a concentration of $10^{-2}M$. The homocystine was finely powdered and passed through a No. 200 gauge sieve.
10. All blood was drawn through No. 19 gauge needles into silicone-coated polystyrene syringes, and plasma was separated in silicone-coated apparatus, avoiding contact with known clot-promoting surfaces. Clotting times were measured in uncoated polystyrene tubes.
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Brush Border Particulates of Renal Tissue

Abstract. Particulates containing a large part of the alkaline phosphatase activity of renal tissue were separated from homogenates and from ribosomal preparations by zonal centrifugation. The particles had a high content of phospholipid and cholesterol that was not removed by treatment with 1 percent deoxycholate. Enzymatic activities concentrated with the particles were the alkaline phosphatase, a peptidase resistant to proteolysis, glucose-6-phosphatase, inorganic pyrophosphatase, and adenosine triphosphatase. The particles accumulated leucine with no stimulation from soluble factors and with inhibition by other amino acids; the accumulation was stimulated by adenosine triphosphate and was not inhibited by puromycin. The particles appear to be derived from the membranes of the brush borders of tubular cells.

Particulates of renal tissue containing alkaline phosphatase and a "resistant" peptidase are found in preparations of renal ribosomes (1). Ribonuclease destroyed the ribosomal particles without effect upon the centrifugal behavior of the enzymatically active particles, but a complete separation from nucleic acids was not achieved in swinging-bucket rotors. The zonal rotors developed by Anderson (2) were found capable of separation of the particulates directly from homogenates of renal tissue (Fig. 1). In the untreated homogenate the particulates distributed with the microsomal fraction but appeared as a sharper band at 38 percent sucrose (by weight) in homogenates treated with 1 percent deoxycholate. Particles in ribosomal preparations with or without pretreatment with ribonuclease were separated sharply from ribosomal material in similar gradients and banded

at 33 percent sucrose (by weight) without magnesium ion and at 38 percent with $0.005M$ magnesium ion.

Similar particles and activities are not found in the ribosomal preparations from rat liver tissue, and the similar activities in the liver tissue are solubilized by the treatment with deoxycholate.

Most of the alkaline phosphatase of the renal homogenate and all that of the microsomal fraction was found in the purified particles. The peptidase was active in the hydrolysis of leucylglycine and leucyl- β -naphthylamide but is distinct from a leucyl- β -naphthylamidase of the microsomal fraction (3). Other activities concentrated with the particles were glucose-6-phosphatase, inorganic pyrophosphatase (4), and adenosine triphosphatase (Mg^{++} activated and Na-K dependent) (5). In the isolation of the particulates from ribosomal preparations, the yield of these activities was

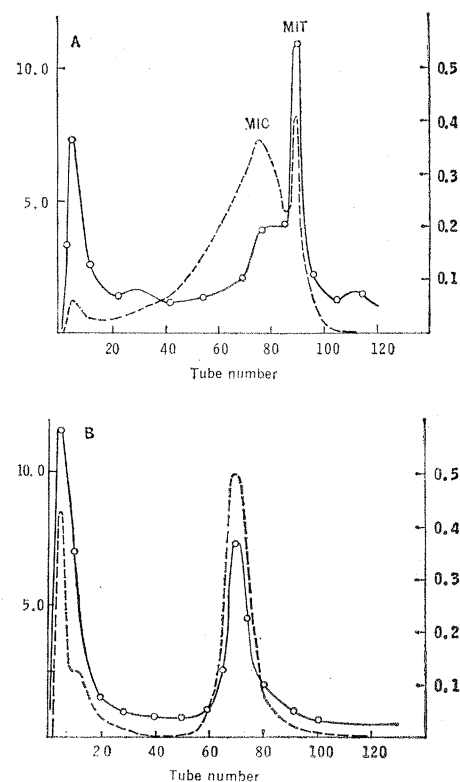


Fig. 1. Zonal centrifugation of homogenates of renal tissue. Only absorbance at $280 m\mu$ (\bigcirc — \bigcirc ; read left ordinate) and alkaline phosphatase activity (— — —; read right ordinate) are plotted; units for both are micromoles per minute per milliliter. Other enzymes used for location were acid phosphatase for lysosomes, cytochrome c oxidase for mitochondria, and acylase I for the soluble fraction. Five grams of fresh rat kidney were homogenized in 15 ml of $0.25M$ sucrose containing $0.005M$ $MgCl_2$ and $0.01M$ tris buffer, pH 8.0, and placed on 1500 ml of 17 to 50 percent (by weight) linear sucrose gradient with a cushion of 150 ml of 55 percent sucrose and an overlay of 57 ml of $0.01M$ tris, pH 8.0, with $0.005M$ $MgCl_2$. Centrifugation was for 2 hours in the B-IV rotor in the Spinco L-4 centrifuge at 20,000 rev/min. Samples of 13 ml each were collected. (A) Untreated homogenate. MIC, microsomes; MIT, mitochondria. (B) Homogenate treated with 1 percent deoxycholate.