The biological role of the extremely repetitive mouse satellite DNA remains a mystery. We have observed it in DNA prepared from the liver of "germ-free" mice and in that prepared from male and female mouse tissues. It has been observed in many tissues and strains (*3*) including European wild mice. It is probably of nuclear origin since our mouse-embryo DNA was extracted from a moderately good preparation of nuclei (*9*), and it is not mitochondrial DNA (*8*). It is an extreme example of the repetition of nucleotide sequences which appears to occur universally in the DNA of higher organisms (*7*).

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4. The word originally introduced was renaturation which applies reasonably well for bacterial or viral DNA from a single species. J. Marmur and D. Lane, *Proc. Nat. Acad. Sci. U.S.* **46**, 453 (1960); J. Marmur and P. Doty, *J. Mol. Biol.* **3**, 585 (1961); J. Marmur, R. Rownd, C. L. Schildkraut, *Prog. Nucleic Acid Res.* **1**, (Academic Press, New York, 1963), p. 275. In the case of mixtures of DNA from related species, or for animal DNA where rematching of the original sequences is unlikely, the word is inapplicable. We use a new word—reassociation—which signifies the stable pairing of DNA strands by virtue of complementary nucleotide sequences, however imperfect the match.
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9. The DNA was prepared from embryos (about 15 days old) of Swiss white mice. The DNA was extracted from a nuclear preparation [B. J. McCarthy and B. H. Hoyer, *Proc. Nat. Acad. Sci. U.S.* **52**, 915 (1964)] by a modification of the method of Berns and Thomas (Abstr., Biophysical Society Meeting, Chicago, February 1962).
10. Quantitative interpretation of the rate of reassociation requires control of the size of the fragments of DNA as well as of the salt concentration and temperature. Passage of a solution of DNA through the needle valve of a French pressure cell [B. J. McCarthy and E. T. Bolton, *J. Mol. Biol.* **8**, 184 (1964)] at a few milliliters per minute with a pressure drop of 800 atm yields a relatively homogeneous population with an average length of about 1200 nucleotide pairs.
11. The SET buffer contained 0.3*M* NaCl and 0.001*M* tris-HCl buffer, *p*H 7.4. The HMP buffer contained 0.001*M* EDTA (ethylenediaminetetraacetate), 0.0075*M* sodium phosphate buffer, *p*H 6.8, total Na^+ concentration 0.013*M*.
12. Under reasonable conditions and except at the later stages, reassociation is a second-order collision-dependent process. It is interfered with by high salt or low temperature, which give excessive secondary structure to the single-stranded regions. For high-molecular-weight DNA the pattern (interspersion in a variety of different orders) of repeated sequences may interfere with the reaction. For sheared DNA without repeated sequences (viral or bacterial), long chains of molecules are formed at the later stages of the reaction by pairing of single-stranded ends of reassociated molecules (*7*). None of these complications affect the conclusions drawn here and probably do not seriously affect the numerical estimate of the length of the repeated nucleotide sequence.
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15. A mixture of polyU and polyA [P. O. Ross and J. M. Sturtevant, *J. Amer. Chem. Soc.* **84**, 4503 (1962)], reassociates much more rapidly than mouse-satellite DNA.
16. The reassociation rate constants (K, liter mol^{-1} $second^{-1}$) at 50°C and 0.08*M* Na^+ for 800 atm sheared DNA are: SV40, $K = 12$; satellite, $K = 180$. Measurements with DNA sheared at 3300 atm and satellite purified on hydroxyapatite yield at 60°C and 0.12*M* Na^+ the following: *Escherichia coli*, $K = 0.040$; satellite, $K = 625$. On the assumption that SV40 contains 6×10^3 and *E. coli* 5×10^6 nucleotide pairs, the calculations give, respectively, 400 and 320 nucleotide pairs as the length of the repeated sequence of the satellite DNA.
17. We thank Drs. B. Hoyer and D. Axelrod of the NIH for discussion, mouse embryos, germ-free mouse DNA, and SV40 DNA; our colleagues at the Department of Terrestrial Magnetism for discussion; and M. Chamberlin for technical assistance.

23 September 1966

Thyrocalcitonin: Effect on Idiopathic Hypercalcemia

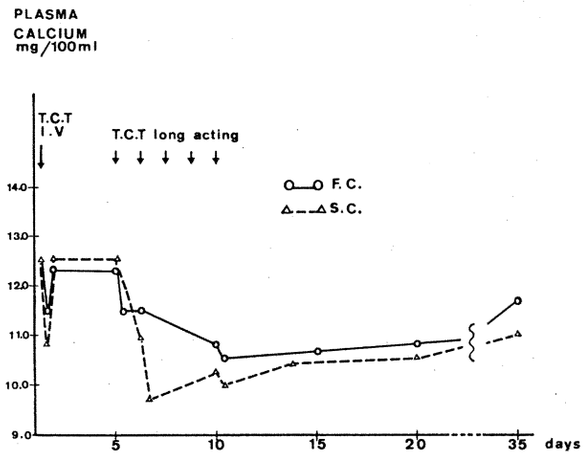
Abstract. *Identical twins having idiopathic hypercalcemia were treated with repeated doses of thyrocalcitonin extracted from porcine thyroid glands. The treatment produced a marked change in the amount of calcium and phosphate in the plasma and in the excretion of urinary calcium.*

Thyrocalcitonin (TCT) causes a decrease in the amount of calcium in the plasma in animals (*1*). When purified porcine TCT is administered to normal subjects (*2*), the effect is rapid and of short duration. However, the long-acting effect of the hormone when administered in repeated doses and in a slowly absorbing vehicle is not known. Evidence for a long-acting effect is provided by our study of 3-month-old male identical twins afflicted with the mild form of idiopathic hypercalcemia, associated with loss of appetite, vomiting, constipation, loss of weight, and increased bone density; hypocalcemic and hypophosphatemic effects were produced.

Thyrocalcitonin was extracted from porcine thyroid glands, purified, and prepared for injection (*2*). One dose of 100 units (*3*) of TCT in a buffered acetate solution was administered intravenously to test the hypocalcemic activity of the preparation (Fig. 1). The maximum response was reached after 1 hour. For example, in the plasma of subject F.C. the concentration of calcium decreased from 12.6 ± 0.25 to 11.5 ± 0.23 mg/100 ml, and that of subject S.C. decreased from 12.5 ± 0.25 to 10.9 ± 0.22 mg/100 ml. After 2 hours the values were: F.C., 11.7 ± 0.23; S.C., 11.0 ± 0.22. Within 4 hours the concentrations of plasma calcium returned to those observed before injection of thyrocalcitonin: F.C., 12.3 ± 0.25; S.C., 12.6 ± 0.25. To overcome this transient effect and to insure a hypocalcemia that would last from 8 to 12 hours, TCT was prepared with a slowly absorbing vehicle (17 percent gelatin), and at days 5 through 10, daily doses of 100 units of TCT were given intramuscularly. The plasma calcium dropped abruptly after the initial injection; it continued to fall during the period of administration and reached normal levels (Fig. 1). After TCT was discontinued there was a gradual increase in the plasma calcium, but the initial hypercalcemia was never attained during the entire period of observation. After 30 days the plasma calcium concentrations were 11.7 (F.C.) and 11.1 (S.C.) mg/100 ml.

Foster *et al.* (*4*) administered, by infusion, high doses of porcine TCT to three patients with hypercalcemia complicated by disseminated malignant disease. The plasma calcium concentrations were lowered but they never became normal.

Hypercalcemia in infancy can be mild or severe (*5*). The identical twins studied had a mild form of the disease, which is attributed by Kenny *et al.* (*6*) to abnormal metabolism of vitamin D. Forfar *et al.* (*7*) have suggested that the severe form of the disease results when the mild form is not treated. Since hypercalcemia can lead to nephrocalcinosis, it seemed particularly important to treat the condition by administering TCT to subjects maintained on a normal diet, rather than by administering cortisone or a low-calcium diet, or both, which would affect growth. Repeated TCT administra-



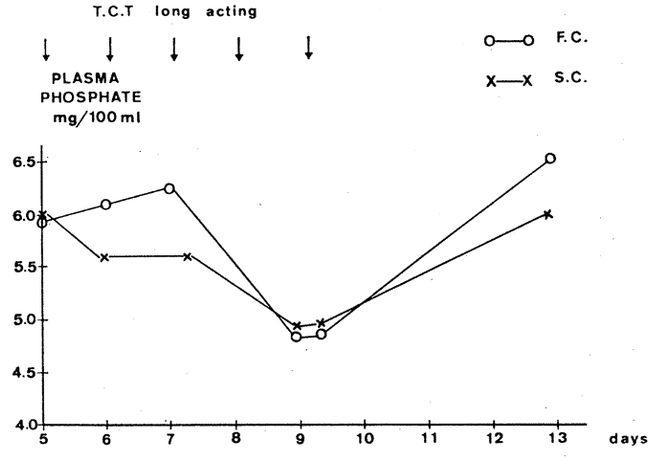


Fig. 1 (left). Plasma calcium concentrations after administration of thyrocalcitonin to two patients with idiopathic hypercalcemia.
Fig. 2 (right). Plasma phosphate concentrations after administration of thyrocalcitonin to two patients with idiopathic hypercalcemia.

tion is very effective in lowering plasma calcium; in idiopathic hypercalcemia this effect seems to be long-acting because it persists 10 days after the last injection. This finding could indicate that bone catabolism is active in this form of hypercalcemia since the mechanism of hypocalcemia produced by TCT is an inhibition of bone catabolism (*8*).

The infants were raised on a formula of evaporated milk from birth; they received daily vitamin D_2 supplements varying from 2000 to 4200 IU until the time of study (3 months of age). When they were admitted to the hospital the plasma calcium values were 17.4 mg/100 ml for F.C. and 16.9 mg/100 ml for S.C. Withdrawal of vitamin D was accompanied by a decrease in plasma calcium levels, which remained stable around 12.6 and 12.4 mg/100 ml until TCT was administered (Fig. 1).

Calcium balance studies, each lasting 5 days, were done before and during the repeated administration of TCT (*9*). In both subjects a decrease in urinary and fecal calcium was observed; the initial positive balance increased in both (Table 1). The balance data can be attributed to relative changes, since short-term balance studies are subject to errors. The important difference in the two periods is the repeated administration of TCT leading to a decrease in urinary and fecal calcium and to an increase in calcium retention. This effect is particularly apparent in subject S.C., whose balance is more positive for a slightly reduced calcium intake. Repeated TCT administration also leads to an increased calcium retention in the growing rat (*10*).

Table 1. Effect of repeated doses of thyrocalcitonin on the calcium balance. Original: values obtained during 5-day balance study before repeated dose regimen; repeated: values obtained during repeated doses.

Dose	Calcium (milligrams per 24 hours)			Balance
	Ingested	Urinary	Fecal	
	Subject F. C.			
Original	794	59	414	+ 321
Repeated	860	42	400	+ 418
	Subject S. C.			
Original	864	48	524	+ 292
Repeated	840	28	455	+ 357

The patients were under observation for 24 days after the last TCT injection; their plasma calcium increased progressively from 10.0 to 10.4 and 11.1 mg/100 ml (S.C.) and 10.7 to 10.9 and 11.7 mg/100 ml (F.C.), and their clinical condition remained favorable. These changes in plasma calcium concentrations do not reflect the variations in the precision of the method since the accuracy is ± 0.2 mg/100 ml. The extent to which the persistence of the lower plasma calcium concentrations was related to thyrocalcitonin administration could be questioned. However, the progressive increase in plasma calcium with time suggests a diminishing thyrocalcitonin effect; in our experience the highest values for normal plasma calcium concentrations in children are below 11.0 mg/100 ml. In the case of F.C. the final value was 11.1, whereas that of S.C. was 11.7 mg/100 ml. Along with this increase in the plasma calcium, urinary excretion of calcium also increased to that observed before administration of TCT—62 mg for F.C. and 52 mg for S.C. per 24 hours.

Hypophosphatemia produced by the administration of TCT occurs in the rat (*1, 11*) but has never been demonstrated in man. It appears from Fig. 2 that TCT produced a dramatic decrease in plasma phosphate concentration in both subjects at the 3rd day and reached its maximum effect at the 4th day. The duration of hypophosphatemia is short compared with the duration of hypocalcemia, since 4 days after the last dose of TCT was administered the concentration of phosphate returned to that observed before treatment.

The management of idiopathic hypercalcemia with a purified and long-acting TCT preparation makes clinical use of the new hormone possible. We have also obtained favorable results in hyperparathyroidism and in hypercalcemia of other origins (*12*).

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precipitation; fecal calcium was measured, after dry ashing, by the same method. Plasma phosphate was determined by the method of Fiske and Subbarrow [*J. Biol. Chem.* **66**, 375 (1925)]. During the two 5-day calcium balance periods urine and stools were collected, the latter being marked by carmine red, and the calcium intake was accurately determined.

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1 August 1966

Adenovirus Multiplication: Inhibition by Methisazone

Abstract. *Methisazone (5 to 40 μM) inhibited the multiplication of types 3, 7, 9, 11, 14, 16, 17, 21, and 28 adenovirus; SV15 (a simian adenovirus) was also inhibited. A study of adenovirus 11 under single-cycle conditions showed that multiplication of the virus was completely inhibited by 30 μM methisazone when addition of the compound was delayed until 13 hours after infection. A survey showed that the structure-activity relations of the action of methisazone against adenoviruses and pox viruses are similar.*

Methisazone (1-methylisatin 3-thiosemicarbazone) will inhibit the multiplication of vaccinia (*1*) and smallpox (*2*) viruses in mice and is effective in the prophylaxis of smallpox (*3*) and alastrim (*4*) in man, as well as in the treatment of eczema vaccinatum (*5*) and vaccinia gangrenosa (*6*). The compound had no apparent effect in mice that had been infected intracerebrally with 15 other viruses, mostly arboviruses (*1*), and it was concluded that the spectrum of antiviral activity of methisazone was extremely limited. In this report we show that methisazone is highly active against certain types of adenovirus and that its spectrum of activity is therefore much wider than had been suspected.

Methisazone was dissolved in the minimum amount of dimethylformamide and added to 900 ml of distilled water; this suspension was autoclaved at 1.7 atm for 10 minutes to effect solution of the compound. To the 900 ml of solution 100 ml of tenfold concentrated Eagle growth medium was then added, which gave a final concentration of 40 μM methisazone. Further dilutions, down to 5 μM methisazone, were prepared in Eagle medium.

In a preliminary study of toxicity, monolayers of HeLa cells in bijou bottles were incubated with 40 μM methisazone for 2 hours; the compound was then washed off, and the cells were infected with adenovirus 11 and incubated for 72 hours. Cell cultures that had not been exposed to methisazone were infected similarly. Cells were disrupted by three cycles of freezing and thawing, and the amount of virus in the supernatant fluid was determined by hemagglutinin titration with patas monkey red cells on perspex plates for 1 hour at 37°C.

In comparison with the controls, there was no depression in the titer of virus from cells exposed to methisazone and we concluded that exposure to 40 μM methisazone did not affect the ability of cells to support multiplication of adenovirus 11. Uninfected HeLa cells were also incubated with 40 μM methisazone for 48 hours, and at the end of this time they appeared normal; when subcultured they grew normally and could be maintained in further passage in the usual way. We concluded that 40 μM methisazone was not toxic for HeLa cells and that this would be a suitable concentration for use in studies of antiviral effect.

Monolayers of HeLa cells, in bijou bottles, 12-ml screw-capped tubes, or in 50-ml prescription bottles, were infected with adenovirus 11 in a multiplicity of around four TCD_{50}'s (tissue culture doses 50 percent infective) per cell after the cultures had been at 4°C or at room temperature for 1 hour to permit absorption to take place. Resid-

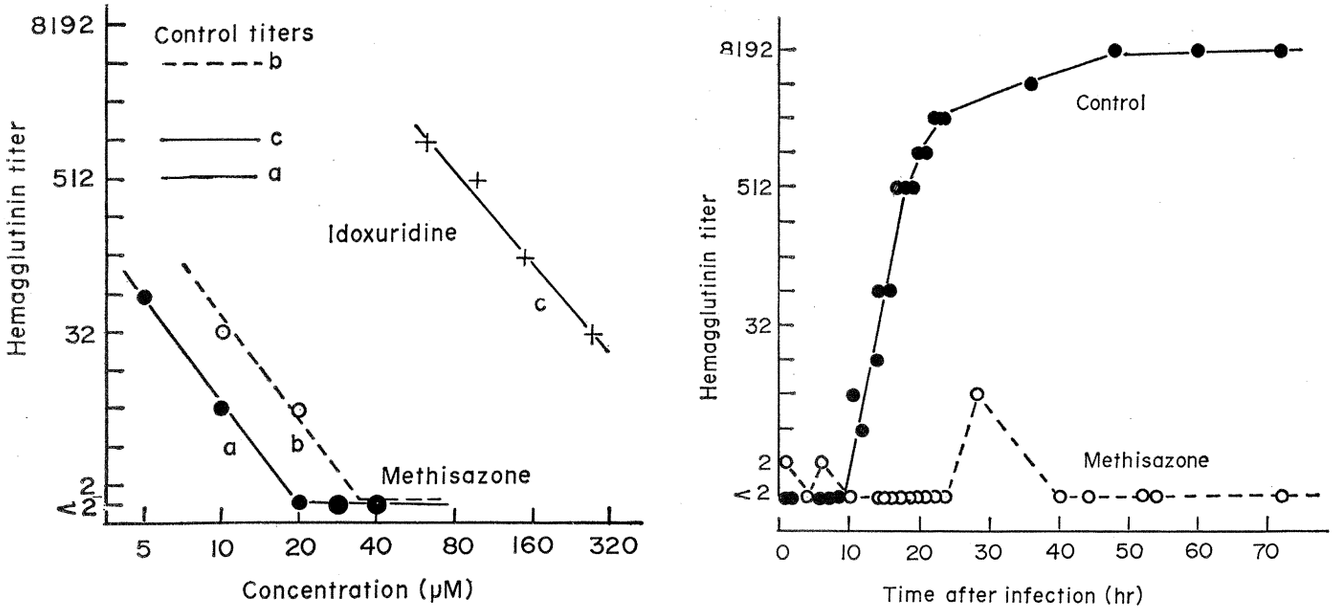

Fig. 1 (left). Dose-response lines of antiviral effect of methisazone (*a*, hemagglutinin production; *b*, infectivity and idoxuridine *c*, hemagglutinin production). Upper lines indicate level of titers in control cultures that did not contain antiviral compounds. Fig. 2 (right). Inhibition by methisazone of the production of hemagglutinin by adenovirus 11 in HeLa cells during a single growth cycle.