at slightly over 3200 cm^{-1}. These bands are present in the mixture but absent in the spectrum of each component. This can be seen by plotting the intensity of these bands against the mole ratio (Fig. 5). The optical densities were obtained by adjusting the transmittance of the pure cyclohexyluracil and pure ethyladenine solutions to have the value zero. The solid circles represent experimental points, and the dashed lines form a smooth curve through them in the upper two graphs of Fig. 5. The solid lines are drawn approximately as tangents to the experimental curve at either end. There is a maximum rise of optical density in a 1:1 mixture of the uracil and adenine derivatives.

The stoichiometry of polynucleotide reactions has been studied in the ultraviolet absorption spectrum by analogous methods (*9*). Data of this type may be used to learn something about the equilibrium constant of the reaction. For example, if the species are interacting fully, then one obtains data which follow the solid tangential lines. The fact that the experimental points fall below the solid line is an indication that the molecules are not all hydrogen bonded into 1:1 dimers. In the lowest curve of Fig. 5, the change in optical density is too small compared to the scatter of the points to demonstrate this.

If data of the type shown in Fig. 5 are sufficiently accurate, it is possible to interpret the figure quantitatively to obtain an equilibrium constant. The data for the 3330 cm^{-1} band suggest a value of the order of 10^3 liters per mole if we assume that the interval between the solid lines and the experimental dashed line represents the unreacted species. However, more accurate data will have to be obtained at the two extremes of the mole-ratio coordinate in order to be certain that the solid lines actually represent the experimental behavior in that region.

Uracil can dimerize with adenine by two different modes of hydrogen bonding. The amino group of adenine can bond with a carbonyl oxygen atom of uracil, but the N—H group of uracil can bond with either nitrogen-1 of the adenine or the nitrogen-7 of the imidazole ring. This was first indicated when polyadenylic acid was found to combine with two moles of polyuridylic acid to form a three-stranded helical molecule (*10*). X-ray diffraction studies of single crystals containing adenine plus either thymine (*11*) or uracil (*12*) derivatives have shown pairing through two hydrogen bonds by way of the imidazole nitrogen (N-7). From our studies we cannot choose between these two types of hydrogen bonding, since both types involve formation of an N—H · · · O hydrogen bond and an N—H · · · N hydrogen bond. However, it is not surprising that in our study of the hydrogen bonding, the monomers form a dimer complex of one adenine and one uracil but not a trimer of one adenine with two uracil derivatives. Entropic effects with small molecules make it very unlikely that the stabilizing energy of the hydrogen-bonded trimer would be large enough to permit formation of detectable amounts of such a trimer.

Although adenine derivatives and uracil derivatives can form hydrogen bonds with themselves in deuterochloroform, they do so only to a limited extent compared to the fairly pronounced association of the adenine and uracil derivatives with each other to form 1:1 hydrogen-bonded pairs. This infrared study provides the first direct evidence that these monomeric molecules are hydrogen-bonding when present together in solution. In view of the nature of the solvent, it is unlikely that there is much stacking of the bases in solution, and therefore the amount of hydrophobic bonding is minimum in this solution.

Our work suggests that there are factors stabilizing the hydrogen-bonded association of adenine and uracil derivatives other than simply the number and type of hydrogen bonds. The self-dimerization of either the adenine or the uracil derivatives involves two hydrogen bonds, as is the case in the adenine-uracil dimer. Nonetheless, the latter is more stable than the former. This suggests that the purine-pyrimidine pair may be stabilized by forces such as dipole-dipole or dipole-induced dipole interactions in addition to the hydrogen bonds themselves.

ROY M. HAMLIN, JR.
R. C. LORD
ALEXANDER RICH
Departments of Chemistry and *Biology*, and *Spectroscopy Laboratory*, *Massachusetts Institute of Technology*, *Cambridge*

References and Notes

1. P. O. P. Ts'o, I. S. Melvin, A. C. Olson, *J. Amer. Chem. Soc.* **85**, 1289 (1963).
2. E. Küchler and H. Tuppy, *Biochim. Biophys. Acta* **80**, 669 (1964).
3. R. Hamlin, thesis, Massachusetts Institute of Technology, June 1963.
4. "Tables of Wavenumbers for the Calibration of Infrared Spectrometers," Commission of Molecular Structure and Spectroscopy, I.U.P.A.C., (Butterworth, Washington, D.C., 1961).
5. R. C. Lord and T. J. Porro, *Z. Elektrochem.* **64**, 672 (1960).
6. D. W. Green, F. S. Mathews, A. Rich, *J. Biol. Chem.* **237**, 3573 (1962).
7. W. Cochran, *Acta Cryst.* **4**, 81 (1951).
8. A. Rich, D. R. Davies, F. H. C. Crick, J. D. Watson, *J. Mol. Biol.* **3**, 71 (1961).
9. G. Felsenfeld and A. Rich, *Biochim. Biophys. Acta* **26**, 457 (1957).
10. G. Felsenfeld, D. R. Davies, A. Rich, *J. Amer. Chem. Soc.* **79**, 2023 (1957).
11. K. Hoogsteen, *Acta Cryst.* **12**, 822 (1959); **16**, 28 (1963).
12. F. S. Mathews and A. Rich, *J. Mol. Biol.* **8**, 89 (1964).
13. Supported by grants from NIH, NSF, and NASA.

7 April 1965

Thyroid and Parathyroid Roles in Hypercalcemia: Evidence for a Thyrocalcitonin-Releasing Factor

Abstract. *Extirpation of either the parathyroids or the thyroid in rats acutely impaired their ability to counteract hypercalcemia induced by exogenous parathormone. Thyroidectomy had the greater effect, and in rats subjected to both parathyroidectomy and thyroidectomy the calcium-lowering mechanism was not further impaired, which suggests that the parathyroids secrete a humoral factor that releases calcium-lowering thyrocalcitonin from the thyroid. This hypothesis reconciles previous conflicting reports.*

While evidence has rapidly accumulated for the presence of a fast-acting calcium-lowering hormone, investigators have arrived at contradictory conclusions as to whether the tissue of origin of such a hormone is the parathyroid (*1, 2*) or the thyroid (*3, 4*). Their conflict is apparent, not real, and all the observations reported heretofore (*1–9*) can be reconciled by hypothesizing the secretion of a humoral thyrocalcitonin-releasing factor (TCRF) by the parathyroid gland. The parathyroid may respond to ambient hypercalcemia and secrete TCRF which has no intrinsic calcium-lowering activity but which is carried by the circulation to the thyroid gland where it releases the stores of thyrocalcitonin, a polypeptide with demonstrated calcium-lowering activity (*10*). We have sought further experimental support for this hypothesis by observing the effect of the specific extirpation of either parathyroid or thyroid tissue, or both, on the ability of a young rat to counteract the prolonged hypercalcemic effect of a large exogenous dose of parathyroid hormone.

Table 1. Response of thyroidectomized and parathyroidectomized rats to the hypercalcemia induced by injections of exogenous parathyroid (PTH) hormone.

Group	Tissue extirpated	Preparation	No. of rats	Calcium content of plasma (mg/100 ml)	
				Before injection	Increase 6 hours after injection*
1A	None	Intact	13	10.86 ± .22	0.32 ± .14
1	None	Parathyroid autografts, sham-operated	27	11.10 ± .15	0.43 ± .08
2	Parathyroid	Parathyroid autografts excised just before injection	24	11.23 ± .09	1.45 ± .11
3	Thyroid	Parathyroid autografts; thyroidectomy 1 day before injection	20	11.22 ± .24	2.88 ± .41
4	Thyroid and parathyroid	Thyroidectomy; parathyroid autografts excised just before injection	17	11.14 ± .24	2.79 ± .22

* Comparisons of groups by Student's t-test: 2 versus 1, $p < .001$; 3 versus 1, $p < .001$; 4 versus 2, $p < .001$.

Cameron and Copp (*5*) injected commercial parathyroid extract into 6-week-old rats immediately after they had been surgically parathyroidectomized or thyroparathyroidectomized. Either procedure increased the concentration of calcium in the plasma by 2 mg per 100 ml, as compared to an increase of only 0.5 mg per 100 ml in sham-operated controls. These authors concluded that the thyroid played no role in the response to hypercalcemia. Recently, Talmage *et al.* (*7*) found that thyroidectomy in itself impaired the ability of rats to handle an exogenous calcium infusion but did not test for a similar impairment from parathyroidectomy alone.

In our experiments we first transplanted the parathyroid glands to the hamstring muscle so that thyroidectomy or parathyroidectomy could be performed without damaging the remaining gland. Parathyroids autografted in rats regain full normal function within 1 week (*11*) and appear morphologically intact even under the electron microscope (*12*). Six-week-old Osborn-Mendel rats thus autotransplanted 2 weeks earlier were randomly assigned to three groups. From each rat 1 ml of blood was obtained by cardiac puncture. Immediately afterward, each was injected subcutaneously with parathyroid hormone (Lilly), 85 units per 100 g of body weight, and 6 hours later was bled in the same manner as before. Group 1 had no extirpative surgery, only sham operations. Intact rats of the same age were tested in the same way as group 1 to control the normalcy of the parathyroids after autotransplantation; these rats were designated group 1A. Rats in group 2 were parathyroidectomized, within 5 minutes before the first blood sample was obtained, by excising the small portion of hamstring muscle containing the autografted glands. Rats in group 3 were subjected to a total surgical thyroidectomy 1 day before the first blood sample was obtained, an interval designed to preclude the potential effects of thyrocalcitonin released by surgical manipulation and of the acidosis (*13*) which frequently occurs as a result of postoperative respiratory distress. Three to five days later, the thyroidectomized rats in group 3 were parathyroidectomized by excision of the autotransplants; immediately afterward these rats were retested and were then designated group 4. The completeness of the parathyroidectomy resulting from the excision of autografts in groups 2 and 4 was assured by measuring the calcium in serum obtained from all rats 3 weeks after surgery when they had been on a low-calcium diet for 10 days. At that time 14 percent of "parathyroidectomized" rats in groups 2 and 4 gave evidence of having accessory parathyroid tissue, in that the calcium content of their serum was more than 8 mg per 100 ml as compared to a mean of 6.09 ± 0.25 for the other rats in group 2 and 5.65 ± 0.25 mg per 100 ml for the other rats in group 4. The data for these rats with presumed accessory parathyroid tissue were omitted. The calcium content of the plasma was determined by the semi-automated EDTA (ethylenediaminetetraacetic acid) method of Copp (*14*).

The results are shown in Table 1. It is evident that intact rats and rats autografted with parathyroids were equally able to counteract to a large extent the hypercalcemic effect of exogenous parathormone, but significantly higher increases in calcium concentrations ($p < .001$) were found in rats lacking *either* the parathyroids or the thyroid glands. Furthermore, in rats both parathyroidectomized and thyroidectomized (group 4), no further impairment of the ability of rats to combat the hypercalcemia was observed. This suggests that the part played by the parathyroid in this response is nullified by the absence of thyroid tissue, as would be the case if the parathyroid produced a TCRF.

The statistically greater increase in the calcium concentration of the plasma after extirpation of thyroid tissue alone, as compared to that after parathyroidectomy alone, may indicate that even in the absence of TCRF there is some output of thyrocalcitonin from the rat thyroid. But the calcium concentrations before the injections of hormone in rats without thyroid glands, groups 3 and 4, were not higher than those with the thyroids present, groups 1 and 2, as might be expected if there was such a continuous output of thyrocalcitonin. Another explanation might be that in the few hours after surgical manipulation and removal of the parathyroid autografts, some TCRF is still circulating and can act through the intact thyroid.

This first demonstration of the need for both parathyroid and thyroid tissue in the normal response to a hypercalcemia in rats, and the apparent mediation of the parathyroid effect by the thyroid, supports the hypothesis of a TCRF and thus reconciles the apparent contradictions of previous reports. According to this hypothesis, Copp and Henze were able to find that the hypercalcemic perfusion of anatomically isolated parathyroid tissue *in situ* (*2*) invariably caused a drop in the total concentration of calcium in the plasma because their animals always had an intact *contralateral thyroid* lobe which responded to the TCRF in the perfusate. MacIntyre *et al.* (*4*) perfused

the parathyroid tissue alone in *totally thyroidectomized* animals and therefore observed no effect. Again, hypercalcemic perfusion of thyroid tissue alone in dogs or sheep has consistently failed to elicit a hypocalcemic response (*1*, *2*), but the simultaneous perfusion of both thyroid and parathyroid in dogs, sheep, and goats (*1*, *2*, *4*, *6*) has consistently produced a drop in the calcium concentration of the plasma. Further, this hypothesis allows for the observation of Copp (*1*) that some extracts of parathyroid glands produced a momentary drop in the concentration of calcium in the plasma of intact dogs and explains their effect as that of extracted TCRF, mediated by the dog's own thyroid tissue.

Care recently found that both the hypercalcemic perfusion of parathyroid tissue alone in some surgically thyroidectomized sheep (*9*) and the hypercalcemic perfusion of surgically isolated thyroid tissue in two pigs (*8*) produced a drop in the plasma calcium. In neither case, however, was the elimination of the other tissue ascertained by the demonstration of subsequent deficiency states or by serial sections of the perfused tissue. In view of our hypothesis it seems desirable that any further perfusion experiments should completely rule out the presence of one or the other tissue in the system.

RUBEN F. GITTES
GEORGE L. IRVIN
Surgery Branch, National Cancer Institute, Bethesda, Maryland 20014

References and Notes

1. D. H. Copp, E. C. Cameron, B. A. Cheney, A. G. F. Davidson, K. G. Henze, *Endocrinology* **70**, 638 (1962).
2. D. H. Copp and K. G. Henze, *ibid.* **75**, 49 (1964).
3. P. F. Hirsch, G. F. Gauthier, P. L. Munson, *ibid.* **73**, 244 (1963).
4. G. V. Foster, A. Baghdiantz, M. A. Kumar, E. Slack, H. A. Solman, I. MacIntyre, *Nature* **202**, 1303 (1964).
5. E. C. Cameron and D. H. Copp, *Proc. Soc. Exp. Biol. Med.* **114**, 278 (1963).
6. M. A. Kumar, G. V. Foster, I. MacIntyre, *Lancet* **1963-II**, 480 (1963).
7. R. V. Talmage, J. Neuenschwander, L. Kreintz, *Endocrinology* **76**, 103 (1965).
8. A. D. Care, *Nature* **205**, 1289 (1965).
9. A. D. Care and W. M. Keynes, *J. Endocrinol.*, in press.
10. P. F. Hirsch, E. F. Voelkel, P. L. Munson, *Science* **146**, 412 (1964); M. A. Aliapoulious, A. Savery, P. L. Munson, *Fed. Proc.* **24**, 322 (abstr. 1067) (1965); C. D. Arnaud, A. Tenenhouse, H. Rasmussen, *ibid.*, abstr. 1066.
11. P. S. Russell and R. F. Gittes, *J. Exp. Med.* **109**, 571 (1959).
12. B. K. Wetzel, R. F. Gittes, S. Spicer, unpublished observations.
13. R. F. Gittes and G. L. Irvin, *J. Surg. Res.*, in press.
14. D. H. Copp, *J. Lab. Clin. Med.* **61**, 1029 (1963).
15. We thank Mary Frances Gibbs for her outstanding technical help.

19 April 1965

Configuration of Inactive and Active Polysomes of the Developing Down Feather

Abstract. *Inactive four-ribosome polyribosomes, insensitive to ribonuclease, have the form of tight symmetrical squares and appear in feather cells during the early and intermediate periods of development. When, on the 13th day of incubation, the inactive polyribosomes become sensitive to ribonuclease and can then synthesize protein, the squares open up so that the four-ribosome polysomes are strung out in the configuration characteristic of functioning polysomes.*

Inactive ribonuclease-resistant polysomes which are converted to long-lived, functioning, ribonuclease-sensitive polysomes are found in chick down feathers during their differentiation (*1*). This conversion takes place during the critical transition period when "keratinization" of the feather begins around the 13th day of incubation of the egg. This is the time when disulfide bonds can be first detected histochemically; the feather first

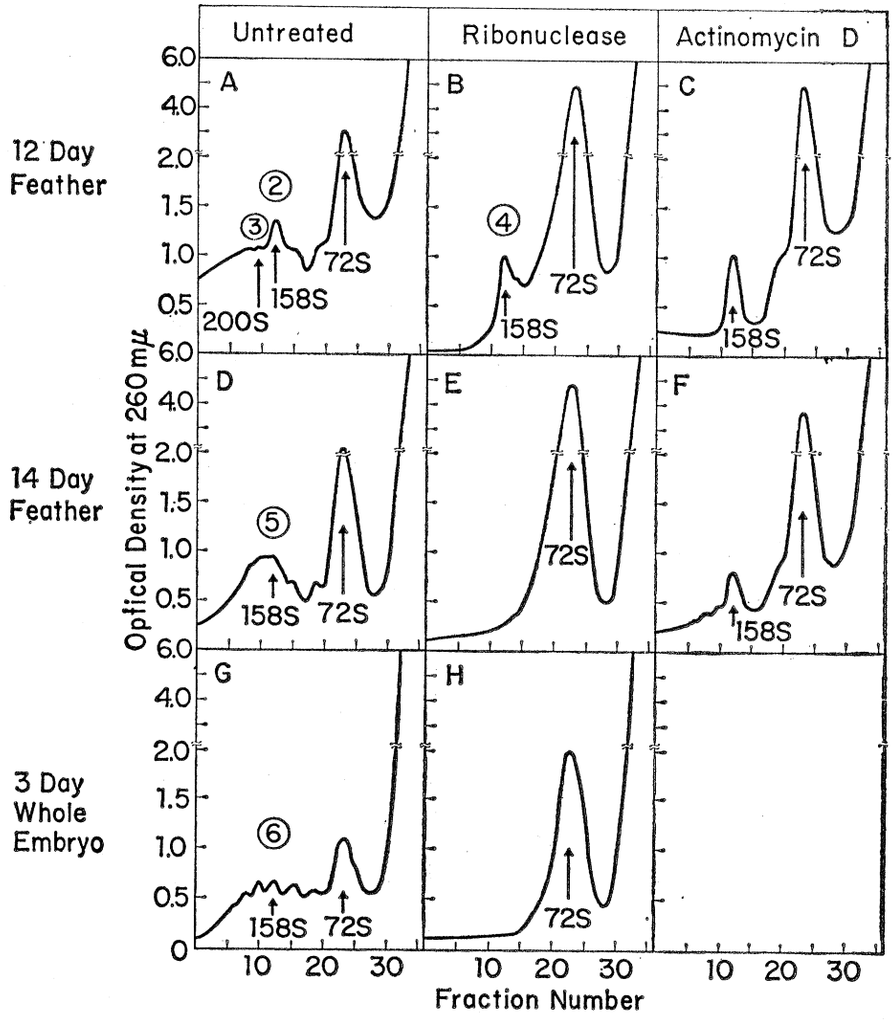

Fig. 1. Optical density at 260 mμ of cytoplasmic extracts of feathers or whole embryos as displayed in sucrose gradients. Numbers refer to electronmicrographs of the fraction. Where indicated, extracts were of feathers treated in vitro with 60 μg of actinomycin D per milliliter of Waymouth medium for 18 hours. Others were untreated, and some untreated extracts were incubated at 4°C for 30 minutes with ribonuclease (4 μg/ml). Extracts were layered on a linear sucrose (15 to 30 percent) gradient and centrifuged for 3 hours at 24,000 rev/min in an SW 25 rotor (model L Spinco). The optical density of gradients was read at 260 mμ in a Gilford continuous-flow spectrophotometer. Treatment of 14-day control feathers (*E*) or 3-day whole embryo (*H*) extracts with ribonuclease resulted in complete loss of optical density due to the polysomes whereas treatment of 12-day feathers dispersed all polysomes except those which sedimented at 158*S*.