Table 1. Ability of supernatant from human blood cells to stimulate growth of bone marrow colonies in vitro. All substances are fractions of the same blood sample. The cells and plasma are from freshly obtained blood and the supernatant is from incubated cells. The supernatant was tested unmodified, after heating at 56°C for 60 minutes, after freezing at −20°C, or after dialysis for 72 hours in Visking tubing against phosphate-buffered saline at 4°C. The number of colonies is expressed as colonies per 100,000 marrow cells. Values represent the mean and standard error of five to ten plates.

Substance	Volume/plate (ml)	Colonies (No.)
Intact blood cells	0.1 (10^6 cells)	0
Sonically disrupted cells	.1 (10^6 cells)	0
Irradiated cells	.1 (10^6 cells)	0
Plasma	.1	0
Supernatant	.1	74 ± 3.3
Heated supernatant	.1	68 ± 2.0
Frozen supernatant	.1	65 ± 2.8
Dialyzed supernatant	.1	68 ± 3.1

the gel was counted by means of an inverted microscope. A colony was defined as a group of more than 50 cells. Intact cells, sonically disrupted cells, or irradiated cells (1000 roentgen) which were not incubated, as well as plasma, were also tested for their colony-stimulating ability (Table 1).

Considerable stimulating activity was present in the supernatant 10 to 14 days after incubation, with nearly all colonies containing 500 to 1500 cells (Table 1). Intact, sonically disrupted, or x-irradiated cells or plasma had no colony-stimulating activity by 7 days. Petri dishes containing intact irradiated or unirradiated leukocytes contained several collections of cells, but none exceeded 50 cells per group.

In two separate experiments, sonically disrupted cells, irradiated cells, or plasma were combined with the supernatant. The number of colonies was reduced by 40 percent by the sonically disrupted and irradiated cells and to a greater extent by the plasma. Irradiated and unirradiated cells washed in McCoy's 5A medium prior to plating had a mild degree of stimulating activity at 7 days, whereas sonically disrupted cells had no activity. The addition of washed irradiated and unirradiated cells to the supernatant resulted in a slight enhancement of colony cell growth. However, the addition of washed sonically disrupted cells resulted in a 40 percent decrease. These observations suggest that both plasma and sonically disrupted cells may be inhibitory to colony cell growth.

Morphologically, colonies contained predominantly granulocytic cells during the first 2 or 3 days and by day 7 were nearly all mononuclear in appearance.

The presence of a colony-stimulating factor in the supernatant of incubated blood cells and only minimum activity in the intact irradiated and unirradiated cells suggests that the substance is not stored within the cells but is produced and released during incubation. Plasma and sonically disrupted cells appear to be inhibitory to colony cell growth, since both reduced the stimulating effect of the leukocyte supernatant.

A study of some of the properties (Table 1) of the stimulating substance reveals that it is not affected by heat or freezing, and is not dialyzable. These properties are in part similar to those described for substances derived from urine (*6*) and other conditioned media (*7*).

Preliminary studies with human bone marrow indicate that colony growth of cells from normal as well as leukemic individuals is stimulated by this substance. Supernatants from incubated leukemic cells also possess colony-stimulating activity.

The finding that the substance produced by incubated human leukocytes will stimulate the growth of granulocytic and mononuclear cell colonies from human or murine bone marrow suggests that this may represent a regulator of granulocytic and mononuclear cell growth. Since some of the properties are similar to those of the substance described in urine (*6*), it seems quite likely that it is produced by leukocytes and excreted in the urine. This substance may serve an important function in regulating granulocytic and mononuclear cell growth in normal individuals as well as in individuals with diseases of the hematopoietic system.

PAUL A. CHERVENICK
DANE R. BOGGS
University of Pittsburgh School of Medicine, Division of Hematology, Pittsburgh, Pennsylvania 15213

References and Notes

1. T. R. Bradley and D. Metcalf, *Aust. J. Exp. Biol. Med.* **44**, 287 (1966).
2. B. Pike and W. A. Robinson, *J. Cell Physiol.*, in press.
3. J. Harris and E. J. Freireich, *Blood* **35**, 61 (1970).
4. J. S. Senn, E. A. McCulloch, J. E. Till, *Lancet* **1967-II**, 597 (1967); J. S. Senn and E. A. McCulloch, *Blood* **35**, 56 (1970).
5. R. Foster, D. Metcalf, W. A. Robinson, T. R. Bradley, *Brit. J. Haematol.* **15**, 147 (1968).
6. W. A. Robinson, E. R. Stanley, D. Metcalf, *Blood* **33**, 396 (1969).
7. D. H. Pluznik and L. Sachs, *Exp. Cell Res.* **43**, 553 (1966).
8. R. G. Worton, E. A. McCulloch, J. E. Till, *J. Cell Physiol.* **74**, 171 (1969).
9. D. H. Pluznik and L. Sachs, *J. Cell. Comp. Physiol.* **66**, 319 (1965).
10. Y. Ichikawa, D. H. Pluznik, L. Sachs, *Proc. Nat. Acad. Sci. U.S.* **56**, 488 (1966).
11. Supported by PHS grant AM-14352 and American Cancer Society grant T-479. We thank Denis Gaza for technical assistance. P.A.C. is a Leukemia Society of America Scholar.

30 March 1970; revised 9 June 1970

RNA and DNA Puffs in Polytene Chromosomes of Rhynchosciara: Inhibition by Extirpation of Prothorax

Abstract. *In the giant polytene chromosomes, gene amplification is made visible by formation of DNA puffs, and gene transcription is made visible by formation of RNA puffs. Ligation of the anterior portion of the larva at the end of the fourth larval instar inhibited the formation of the DNA puffs that normally develop at this stage and caused regression of RNA puffs.*

Synchronous development of sibling larvae of *Rhynchosciara angelae* permits a detailed analysis of the sequential pattern of puffs which occur during development (*1*). During the first three larval instars numerous and specific chromosomal puffs occur, but in general these puffs are small (*2*).

At the prepupal stage, that is, in the late fourth instar, when the larvae gather together to form a colonial cocoon, a new series of chromosomal puffing ensues, and the puffs enlarge greatly (*1, 2*). Principal among these puffs are the DNA puffs where, besides an enhanced RNA synthesis, large amounts of DNA accumulate in specific regions of the giant chromosomes (*1–4*).

Inhibition of puffs in cells under different experimental and pathological conditions has been observed by a number of investigators (*5*). We shall show here that the DNA puffing is inhibited by ligation of the front part of the larvae just behind the brain. This is consistent with the interpretation that an interruption of the flow of brain hormone from the neurosecretory cells to the prothoracic gland will block

the induction of gene activation (*6*).

Rhynchosciara angelae were maintained at 21°C for several generations in the laboratory. The original stock was collected at Cidade da Criança, Vila Atlantica, State of São Paulo, Brazil. At the end of the fourth larval instar, after feeding had stopped, sibling larvae in synchronous development were separated into two groups of 50 larvae each. One of the groups was used as a control; the larvae of the other group were used for ligation experiments.

Three separate experiments were carried out: (i) Larvae were ligated with cotton thread behind the brain between the first and second segments. In order to minimize infections, ligation was followed by cauterization of the part anterior to the ligature. (ii) Larvae were ligated anterior to the brain between the head and the first segment and then cauterized in front of the ligature. (iii) The surface ectoderm was cauterized without ligation.

Larvae ligated ahead of the brain and those only cauterized behaved like the control larvae, so we shall limit our discussions to the experiments involving ligation being the brain. The experiments described here were continued for 4 days after ligation.

Stained preparations of the anterior region of the salivary glands were (*1*) made every day with experimental larvae and controls. The larvae were dissected in a saline solution, and the salivary glands were fixed for 10 minutes in Carnoy's fixative (ethanol and acetic acid, 3 : 1). The glands were removed from the fixative, placed in a drop of a mixture of glacial acetic, 85 percent lactic acid, and distilled water in the proportion of 9:5:6 to which a small amount of 1 percent lactic–acetic orcein was added. The salivary glands were squashed in this solution.

In particular, we analyzed three DNA puffs: puff 2 of chromosome B and puffs 3 and 8 of chromosome C. Two RNA puffs were studied, namely puffs 4 and 5 in chromosome C.

One of the largest of the DNA puffs, DNA puff 2B (*1*), appeared in the control larvae 1 day after the experimental larvae were ligated (Table 1). This puff developed into an enlarged condition in the control larvae over the 4 days of the experiment (Fig. 1). The ligated larvae exhibited a beginning of puffing at the 2B region in some cells (Table 1), but these incipient puffs regressed. A single larva exhibited incipient 2B puffs on the 4th day. It is possible that this larva was at a little more advanced stage than the others when the ligature was made, or that the ligature was not completely effective.

Table 1. Percentage of 2B puffs at the three stages—none, incipient, or full—in the control and in the ligated larvae.

Time after ligation (days)	Treatment	Puff 2B in three sizes (%)			Chromosomes studied (No.)
		None	Incipient	Full	
0	Control	100.0			82
1	Control	32.4	32.4	34.1	120
1	Ligated	77.2	22.8		136
2	Control			100.0	104
2	Ligated	100.0			90
3	Control			100.0	148
3	Ligated	100.0			180
4	Control			100.0	159
4	Ligated	97.1	2.9		138

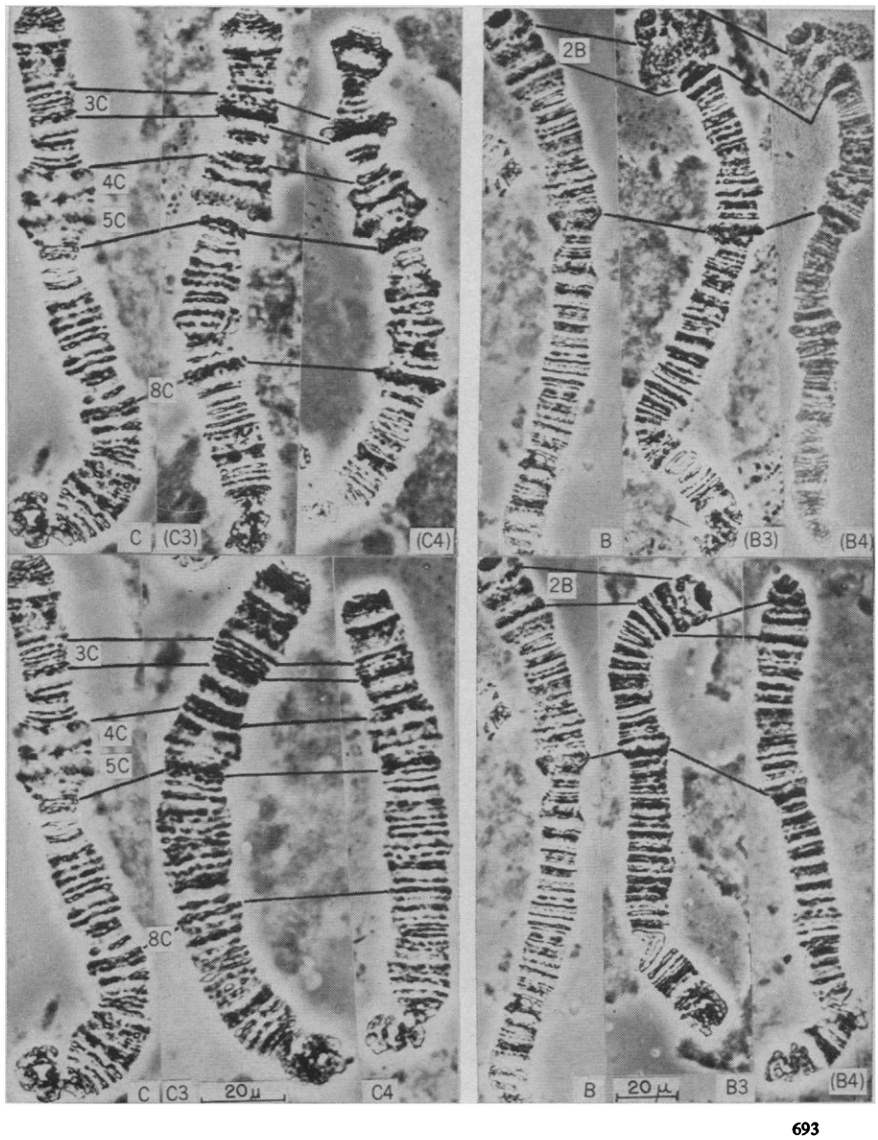

Fig. 1. (Top) Controls. Chromosomes B and C from the control larvae and their conditions 3 (B_3 and C_3) and 4 (B_4 and C_4) days after the experiment was begun. (Bottom) Experimental. Chromosomes B and C from the control larvae before ligation, and their conditions 3 (B_3 and C_3) and 4 (B_4 and C_4) days after ligation.

In the control larvae, the regions 3C and 8C became progressively thicker due to an increase in the DNA content. It is known that 3C after this stage becomes the largest of the DNA puffs in *Rhynchosciara angelae* (*1*). No change took place in the puffs of those larvae that had the ligature (Fig. 1).

The RNA puffs 4C and 5C were already well developed when the larvae were ligated. The puffs were maintained in an enlarged condition in the controls, but regressed in the ligated larvae (Fig. 1). The DNA puff 7B is marked by a line on the figure. It appears that this puff did not regress nor increase, but its DNA content did increase in the control. Other small RNA puffs can be seen in Fig. 1. These generally increased in the controls and declined in the ligated larvae.

It is clear that ligation of larvae behind the brain results in both a lack of DNA and RNA puff induction and a decline in the size of RNA puffs that had already been induced. Thus, we deduce that the ligation is responsible for blocking two different types of events: gene amplification by an increase of DNA (*3*, *7*) and the maintenance of the transcription of RNA by DNA.

J. M. Amabis
Dulce Cabral
Departamento de Biologia Geral, Universidade de São Paulo, Caixa Postal 8105, São Paulo, Brazil

References and Notes

1. M. E. Breuer and C. Pavan, *Chromosoma* **7**, 371 (1955).
2. H. L. B. Guaraciaba and L. F. A. Toledo, *Arquiv. Inst. Biol. (São Paulo)* **35**, 89 (1968); ———, *Rev. Bras. Biol.* **27**, 321 (1967).
3. A. Ficq and C. Pavan, *Nature* **180**, 983 (1957).
4. G. T. Rudkin and S. L. Corlette, *Proc. Nat. Acad. Sci. U.S.* **43**, 964 (1957).
5. R. Goodman, J. A. Goidl, R. M. Richart, *ibid.* **58**, 553 (1967); H. V. Crouse, *ibid.* **61**, 971 (1968); C. Pavan, A. L. P. Perondini, T. Picard, *Chromosoma* **28**, 328 (1969); L. C. Simões and J. M. Amabis, *Ciência e Cultura* **21**, 244 (1969).
6. D. Bodenstein, *Ergeb. Biol.* **13**, 174 (1936); A. Krishnakumaran, H. O. Oberlander, H. A. Schneidermann, *Nature* **205**, 1131 (1965); W. S. Herman, in *Metamorphosis*, W. Etkin and L. I. Gilbert, Eds. (Appleton-Century-Crofts, New York, 1968), pp. 107–141.
7. C. Pavan and M. E. Breuer, in *Symposium on Cell Secretion*, G. Schreiber, Ed. (Univ. of Minais Gerais, Belo Horizonte, Brazil, 1955), p. 90; C. Pavan, *Proc. Int. Congr. Genet. 19th* **1**, 321 (1959); R. C. von Borstel, *Amer. Zool.* **3**, 87 (1963); H. V. Crouse, *Proc. Nat. Acad. Sci. U.S.* **61**, 971 (1968).
8. We thank Dr. L. C. G. Simões for his skillful orientation; Prof. A. B. da Cunha and Prof. R. C. von Borstel for the English translation and suggestions; Drs. J. S. Morgante, R. Basile, H J. Targa, and A. L. P. Perondini for reading the manuscript; Misses Ilze L. Jorge and Maria do Carmo Rabello for typing; Fundação de Amparo à Pesquisa do Estado de São Paulo and Conselho Nacional de Pesquisas for the financial support.

27 January 1970

Circadian Rhythms in Human Heart Homograft

Abstract. *The electrocardiogram and cardiotachogram of a patient with a human heart transplant has been recorded for 72 hours. Within the donor P-QRS-T complex, one can identify the P waves emanating from residual sinoatrial heart tissue of the recipient. The recipient P waves are independent of the donor complexes. A clear circadian rhythm (23.4 hours) in heart rate is maintained for both donor and recipient tissue, the donor complexes preceding by a phase shift of 135 minutes the complexes of the recipient heart tissue. Both tissues display clear morning and evening minimum and maximum rates paralleling activity and lighting cycles.*

Although circadian rhythmicity of the human heart has been demonstrated in normal subjects (*1–3*), human cardiac transplantation has presented a unique opportunity for study of the rhythmicity of the totally denervated human heart. The surgical procedure devised by Lower and Shumway includes removal of all of the recipient's diseased heart, with the exception of that posterior portion of the right atrium which serves as an anchoring bed for the donor heart, and which also contains the sinoatrial (SA) node (*2*). This specialized cardiac tissue provides the rhythmic pacemaker impulse which stimulates normal cardiac contraction and initiates each beat. The donor heart, with its own intact SA node and intact conduction system, is then sutured to the recipient's residual cardiac tissue. Thus, only a suture line separates the two impulses, one arising from each SA node, as recorded by the presence of two distinct P waves (Fig. 1). In a study of such tracings, it is clear that the ventricles of the newly implanted donor heart beat only in response to the firing of the donor SA node, so that the electrical activity of the recipient cardiac tissue appears on the electrocardiogram as an ectopic P wave, normally firing at a different rate from that of the intact donor heart. The recipient SA node thus retains its own intrinsic autonomic innervation, whereas the implanted heart possesses no neural connection to the recipient's autonomic nervous system, and is thus "denervated." [Recent studies, however, have demonstrated intact neural fibers within the donor heart after transplantation (*3*).] Using special electrocardiographic recordings, we studied the rhythmicity

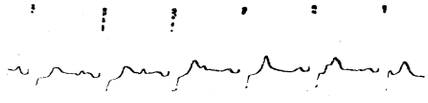

Fig. 1. Lead 2 of the electrocardiographic tracing of donor heart homograft, showing two distinct P waves.

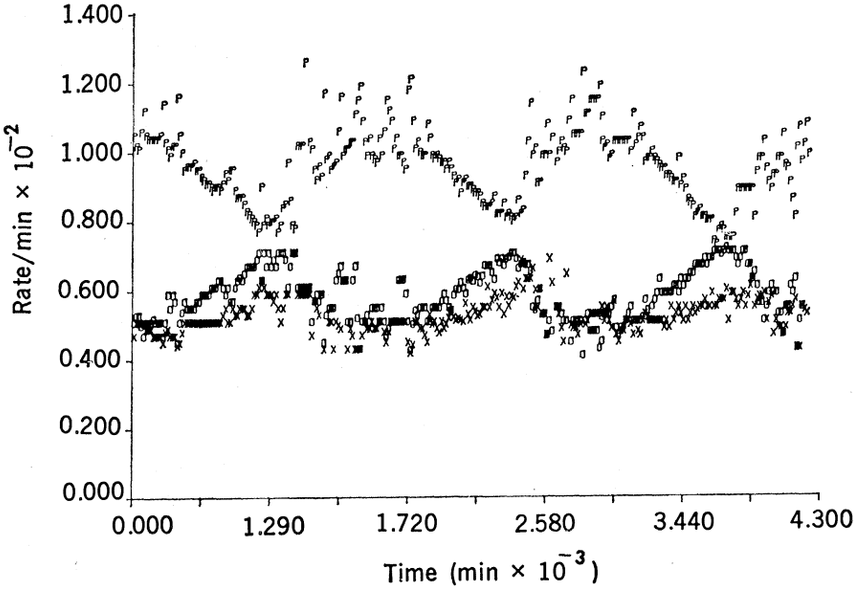

Fig. 2. Heart rate (upper trace, labeled *P*) and P-P interval (lower trace, labeled *O*) of donor heart, and P-P interval of recipient tissue (lower trace, labeled X). The exact reciprocal relationship between heart rate and P-P interval of donor heart is clearly seen. The recipient P-P interval shows a similar period, but the phase shift is evident.