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.

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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

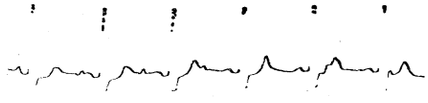

Fig. 1. Lead 2 of the electrocardiographic tracing of donor heart homograft, showing two distinct P waves.

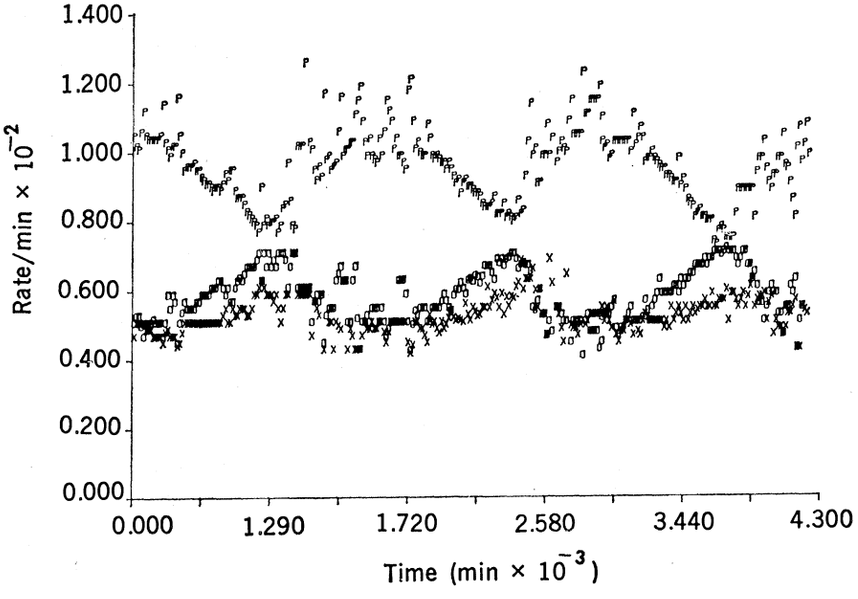

Fig. 2. Heart rate (upper trace, labeled *P*) and P-P interval (lower trace, labeled *O*) of donor heart, and P-P interval of recipient tissue (lower trace, labeled X). The exact reciprocal relationship between heart rate and P-P interval of donor heart is clearly seen. The recipient P-P interval shows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

of both donor and recipient nodal tissue.

A 50-year-old male patient was studied 32 days after receiving a heart homograft. The patient's electrocardiogram was monitored for 72 hours with a Beckman-Offner two-channel recorder and a-c–d-c coupler. Heart rate was computed by means of a Beckman beat-by-beat cardiotachometer and recorded on the other channel. The two tracings were recorded at a paper speed of 1 mm/sec. To obtain data for analysis, we increased the paper speed every 15 minutes to 25 mm/sec for a period of 30 seconds. When awake during these 30-second periods, the patient was asked to lie quietly in bed. The patient's activity, medications, and visitors were monitored. The data submitted for computer analysis were derived as follows: The average heart rate for each 30 seconds was determined visually. For the donor heart, five intervals between P waves (P-P intervals) from each 30 seconds were measured manually; the values were averaged and converted to seconds by means of the equation 1.0 mm equals 0.04 second. For the recipient heart, five SA node P-P intervals recorded during the same 30 seconds were similarly treated. The heart rate in beats per minute and P-P intervals in seconds were tabulated and plotted so that trends could be observed (Fig. 2); subsequently, autocorrelation, cross-correlation, covariance, and computation of power spectral density were determined from data so that comparisons could be made between donor rate and donor P-P interval, between donor rate and recipient P-P interval, and between donor and recipient P-P intervals.

The results indicate a clear, circadian rhythmicity of denervated donor human heart throughout the 72 hours of study. The resolution of the statistical treatment allows one to identify a period length of 23.4 hours for this primary, essentially circadian, rhythm. As expected, a reciprocal relationship exists between the rate of the donor heart and the length of the P-P interval of the donor heart (Fig. 2). Persistent electrical activity of the recipient's residual atrial tissue with an electric signal consistent with the SA nodal depolarization (P) wave was seen in all electrocardiograms. A circadian rhythm was observed in the electrical signal emanating from the recipient SA nodal tissue, this also displaying a period length of 23.4 hours. There was an approximate 135-minute phase shift between donor rhythmicity and recipient rhythmicity

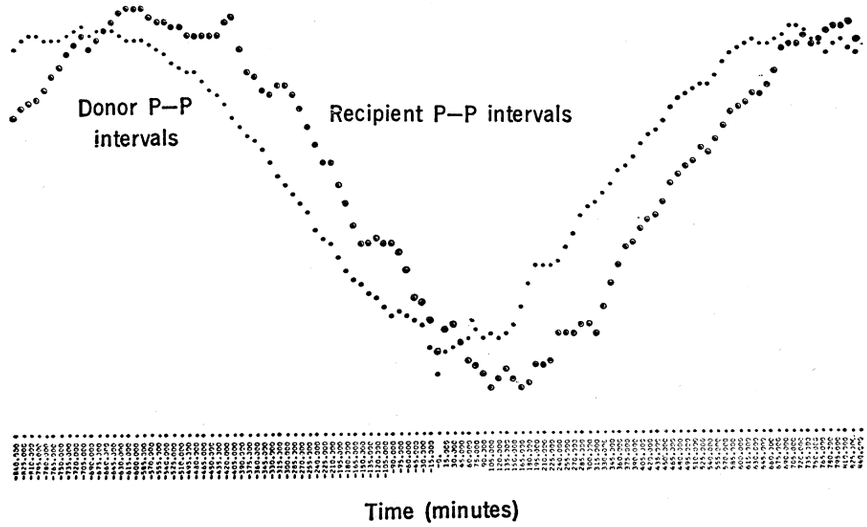

Fig. 3. Superimposed autocorrelation plots of depolarization rate of donor and recipient SA nodes for 24 hours. The period of the donor heart, although similar to that of the recipient tissue, precedes the recipient in phase by approximately 135 minutes.

consonant with the visual tracing showing the nodal tissues to have changes in P-P interval over time which were out of phase with each other, the donor tissue always achieving maximum and minimum rates prior to the recipient tissue (Fig. 3). It is significant that the denervated donor heart displayed maximum and minimum rates in late afternoon and early morning, respectively, paralleling the activity and lighting schedule of the patient, and consistent with what has been observed in normal human hearts under similar activity and lighting schedules (*4*).

The physiologic factors responsible for the observed circadian rhythmicity of the normal heart are unknown. Alterations of circadian rhythm of heart rate can be manipulated by subtle changes in environmental cues, such as light and cycles of work and rest (*5*). Such exogenous influences have been postulated to work through biological oscillators located in (i) the central nervous system, (ii) autonomic effectors of humoral release, or (iii) the specialized neural myocardial cells of the SA node (*6*).

Berlyne *et al.* have recently shown the presence of a diurnal rhythm in rates of water and electrolyte excretion in renal homografts from cadavers (*7*). Our studies here demonstrate that the denervated donor heart maintains a similar circadian rhythm. The cadaver kidney displayed a phase shift of 180° from the preoperative rhythm of electrolyte excretion, whereas the cardiac tissues described here maintain a rhythm closely entrained to environmental cues, and shifted in phase by only 30°. It has also been demonstrated that the allografted human heart, in contrast to normal controls, increases its rate after exercise only after a lag period of approximately 2 minutes (*8*), which suggests that it is responding to circulating catecholamines, and not to direct innervation.

The difference in phase between nodal rhythms of donor and recipient tissues gives added weight to the concept of autonomy and absolute denervation of the donor heart. In subsequent studies in this patient and others, the difference in phase has invariably been present, and no spillover of neural excitation and spread of conduction has been observed. It is possible that with time, or in other species (dog) (*9*), one may see restitution of neural connections to the donor heart at the site of the anastomoses.

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Cell-Mediated Immunity Shown by Lymphocytes from the Respiratory Tract

Abstract. *Cell-mediated immunity, as evidenced by inhibition of macrophage migration in the presence of antigen, was associated with lymphocytes obtained from the respiratory tract of guinea pigs immunized by dinitrophenylated human immunoglobulin G in nose drops, but not from those immunized parenterally. However, splenic lymphocytes from parenterally immunized animals inhibited macrophage migration while those from locally immunized animals did not.*

Lymphoid cells of the respiratory tract can produce antibody after local antigenic stimulation (*1*). This humoral response, thought to be independent of systemic antibody production, is characterized by production of antibodies belonging to the immunoglobulin A (IgA) class. However, there has been no report of a cell-mediated immune response in external secretions. The increasing awareness that cell-mediated immunity functions in a variety of defense mechanisms and disease processes suggested to us that, in addition to the induction of antibody synthesis, there might be lymphoid cells present which could specifically react with antigen and effect those reactions associated with cell-mediated immunity.

Experiments were devised to study the development of sensitized lymphocyte (*2*) production in the respiratory tract and to compare these lymphoid cells with those present in the spleens of animals after administration of antigen, either locally (nose drops), or by injection of antigen in complete Freund's adjuvant into the footpad. The results suggest the existence of cell-mediated immunity in cells obtained from the respiratory tract after local antigenic stimulation. After systemic immunization, sensitized lymphocytes could be demonstrated in the spleen but not in cells from the respiratory tract.

Twelve adult Hartley guinea pigs (approximately 400 g) were immunized with 50 μg of dinitrophenylated human immunoglobulin G (DNP-HGG) in complete Freund's adjuvant injected into the rear footpads. Another group of 12 animals was lightly anesthetized (with sodium pentobarbitalum injected intraperitoneally) and given approximately 200 μg of the same antigen in 0.15 ml of phosphate-buffered saline nose drops. Three animals from each group were exsanguinated 11, 15, 19, and 26 days after administration of antigen, and the spleens, tracheae, bronchi, and lungs were removed. The lower respiratory tracts were externally washed, and 5 ml of sterile Eagle's medium was instilled into the main-stem bronchi in 1-ml portions. The cell suspension was aspirated and then centrifuged at 1500 rev/min for 5 minutes; the resulting pellet was resuspended and washed twice with Eagle's medium containing 10 percent inactivated calf serum. The final suspension contained between 3.1×10^6 and 5.0×10^6 lymphocytes and 4.6×10^6 to 7.5×10^6 macrophages per animal.

Table 1. The effect of local and systemic immunization on the development of sensitized lymphocytes. Lymphocytes were obtained after immunization with nose drops (200 μg DNP-HGG) or with 50 μg of DNP-HGG in Freund's adjuvant injected into the footpads.

Time after stimulation (days)	Mean macrophage migration inhibition by lymphocytes after immunization by:	
	Nose drops	Footpads
	Lymphocytes from spleen	
11	5.1 ± 4.5*	57 ± 6.9
15	5.0 ± 4.2	58 ± 3.8
19	10.0 ± 4.7	62 ± 9.6
26	5.0 ± 3.2	11.5 ± 4.0
	Lymphocytes from bronchial washing	
11	60 ± 7.7	0
15	59 ± 9.0	31 ± 3.5
19	26 ± 6.5	0
26	8.2 ± 5.5	0.5 ± 0.2

* Mean of six determinations and standard error of the mean.

The spleens were homogenized by hand in a glass grinder containing modified Eagle's medium containing fourfold amounts of amino acids and vitamins. The cell suspension was centrifuged at 1500 rev/min for 5 minutes, and the sediment was resuspended and allowed to stand for 1 hour at 4°C. The cells remaining in the supernatant were sedimented by centrifugation at 1500 rev/min for 5 minutes and resuspended in 0.83 percent sterile NH_4Cl to induce erythrocyte lysis. Thirty minutes later the remaining lymphoid cells were again sedimented and washed twice in Eagle's medium containing 10 percent inactivated calf serum. The number of viable lymphocytes distinguished by the Trypan blue exclusion test were determined with a hemocytometer.

Lymphocyte preparations from the spleens and bronchial washings of the same animals were assessed for the presence of specifically sensitized lymphocytes by estimation of their capacity to inhibit macrophage migration in the presence of DNP-HGG (*3*). Cells from normal guinea pig peritoneal exudate were harvested aseptically 48 hours after the administration of 20 ml of paraffin oil intraperitoneally (*4*). The average number of macrophages thus obtained was 1.3×10^8 per animal. Splenic lymphocytes were mixed with peritoneal macrophages in Eagle's medium containing 10 percent inactivated calf serum or in the same medium containing 300 μg of DNP-HGG per milliliter, so that the final suspension contained 5×10^6 lymphocytes and 25×10^6 macrophages per milliliter. Capillary tubes were filled from each cell suspension and sealed with paraffin wax. The filled tubes were centrifuged, cut cleanly at the cell interface, and mounted into wax-paraffin chambers filled with Eagle's medium containing 10 percent inactivated calf serum, with or without the addition of 300 μg of DNP-HGG per milliliter. The chambers were then stored in a CO_2 incubator at 37°C for 48 hours. The extent of macrophage migration was assessed at 24 and 48 hours by projecting the migration areas onto a glass screen and then tracing and weighing them. The effect of antigen on migration was assessed by calculating the migration in the presence of antigen as a percentage of that obtained in its absence.