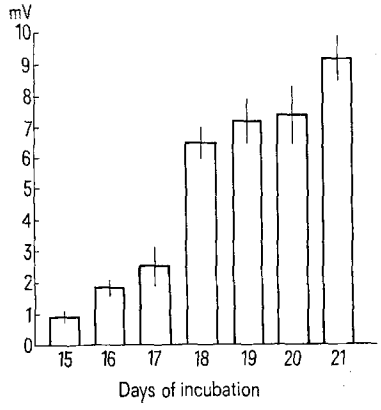
Development of the Steady Potential Differences of the Eyeball in Chick Embryos

The electrical activity of the optic afferent system in chick embryos is established at the break between day 17 and 18 of incubation[1-3]. The onset of functional activity of this system is based on synchronized maturation of pheripheral receptor and of projection centers in optic tectum. We performed this study of the development of DC potential of eyeball in chick embryos with the aim of further description of developmental stages of peripheral optic receptor.

Material and methods. 70 chick embryos (10 per day of incubation) of white Leghorns at the age from day 15 to 21 incubation were used. No anesthesia was applied. The head of embryo was fixed in a special stand. Both eyelids and nictitating membrane were removed without bleeding. The fine wick calomel electrodes were used: one electrode was placed in the center of corneal surface, the other one on the external surface of eyeball at the equatorial level some mm behind the corneal limbus. Steady potential differences were read out from the scale of compensation millivoltmeter with cathode follower input (grid current $< 5\times10^{-11}$A, input impedance $> 2\times10^{10}$ohm). The values of steady potential were measured in 30-sec intervals during 5 min after arrangement of electrodes. The experiments were carried out in darkness in an incubator at 37 °C.

Results. The DC potential of eyeball increased in 3 stages. The first significant increase occured between day 15 and 16 of incubation: from 0.92 $\pm$ 0.21 mV to 1.87 $\pm$ 0.23 mV ($p < 0.01$). The second stage was between day 17 and 18 of incubation: the DC potential increased from 2.57 $\pm$ 0.63 mV to 6.51 $\pm$ 0.46 mV ($p < 0.001$). Then the steady potential developed gradually to the value of 9.26 $\pm$ 0.76 mV in 21-day-old embryos, but the significant difference was between day 18 and 21 only ($p < 0.002$) (Figure).



Developmental values of steady potential differences of the eyeball in chick embryos. Columns: mean $\pm$ s.d. ($n = 10$ per day of incubation).

The developmental differences were manifested also in the stability of DC potential of eyeball within 5 min of measurement. It was shown that the stability developed in the same stages as the value of DC potential. The steady potential differences decreased in 15-day-old embryos within 5 min of measurement by 85 % of initial value, in 16- and 17-day-old embryos by 60.4 %, whilst in 18 to 21-day old embryos on anaverage by 22.5 % of initial value only.

Discussion. The steady potential differences of the eyeball may be derived mainly from 3 sources: from the activity of ciliary body[5,6], from the activity of retinal pigment cells[7] and from retinal neuronal elements[8], which are the developmental derivates of the optic tectum.

The main critical point of the DC potential of eyeball development was around day 17 of incubation similarly to the development of steady electrical properties of brain parts in chick embryos[4,9]. This critical moment is in full accordance with the functional onset of optic afferent system at the break between day 17 and 18 of incubation[3].

The development of DC potential of the eyeball in chick embryos is a further proof for the consideration that the peripheral part of optic afferent system undergoes around day 17 of incubation essential changes of maturation which ensure the onset its functional activity[10].

Résumé. Le potentiel D.C. mesuré sur la surface du globe oculaire de l'embryon de poulet croît à partir du 15ème jour de 0.92 $\pm$ 0.21 mV jusqu' à 9.26 $\pm$ 0.76 mV au 21ème jour l'incubation. L'augmentation maximum se situe entre le 17ème et 18ème jour (2.57 et 6.61 mV). Elle correspond à la mise en jeu de l'appareil visuel afférent. La stabilité du potentiel D.C. du globe oculaire est directement proportionnelle à sa valeut.

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Chronotropic Changes due to Pericardial Distension in Isolated Frog Hearts Perfused in situ

The influence of mechanical strech induced by changing the intraluminal pressure in the chamber containing the cardiac pacemaker, on the chronotropic response of amphibian and mammalian hearts, has been extensively investigated (PATHAK[1-5]) and it has been demonstrated that mechanical stretch acts as a fundamental stimulus for intrinsic autoregulation of pacemaker (PATHAK[6,7]). This work was prompted by the consideration that pacemaker cell configuration and fibre-lenght is also likely to be altered by extramural pressure changes. Since this aspect of pericardial tamponade has not been investigated so far, a preliminary report would be stimulating.

Material and methods. Hearts of decapitated frogs were perfused in situ through the posterior vena cava at a constant optimal pressure of 3 cm H_2O (PATHAK[1-3]) regulated by an overflow device, the velocity of flow being

kept constant with the help of a specially designed cannula connected to a reservoir containing Ringer's solution (NaCl 102 mM; KCl 1 mM; $CaCl_2$ 1 mM and $NaHCO_3$ 1 mM). The pH of Ringer's solution was about 7.6. The experiments were done at room temperature. Pericardial distension was conducted in graded steps by injecting Ringer's solution taken from the perfusion reservoir in 0.5 ml or 1 ml increments from a syringe connected to a polythene catheter placed in the pericardial cavity near the atrioventricular groove and tied securely. Sudden distension was also conducted in some hearts by injecting larger volumes (2 to 5 ml) in one shot. To exclude the possibility of ionic involvement, the pericardial distension was also conducted with air injection in some experiments. The system was checked for any possible leak before and after each experiment. The heart rate was monitored on electronic rate meter triggered by the ventricular complex of the electrocardiogram which was recorded on heat sensitive paper.

Results. The results of experiments on 26 hearts (Table) are considered here. In all hearts, rate changes were observed during pericardial distension. In 12 hearts the rate increased in a stepwise manner as more and more fluid was injected into the pericardial sac. Out of these 12 hearts, 11 showed maximum rate increase when 1 to 4 ml of fluid had been injected. The increase in the heart rate ranged from 18 to 148%, with an average of 53%. After the peak heart rate had been reached, further injection of fluid reduced the rate progressively to below control values. Thus in these hearts there was a phase of initial acceleration followed by a phase of deceleration (Figure 1). The time sequence of positive chronotropic effect showed that the peak rate was reached with a time lag of 1 to 4 min after which the rate stabilized to a slightly lower value (Figure 1D inset top right).

In the remaining 14 hearts (54%) the acceleration phase was absent. The heart rate decreased progressively as more and more fluid was injected (Figure 2). The decrease in the heart rate ranged from 14 to 48%, with an average of 28%. There was no relation between the nature of response and the initial heart rate.

Electrocardiographic record showed significant changes in impulse conduction on distending the pericardium

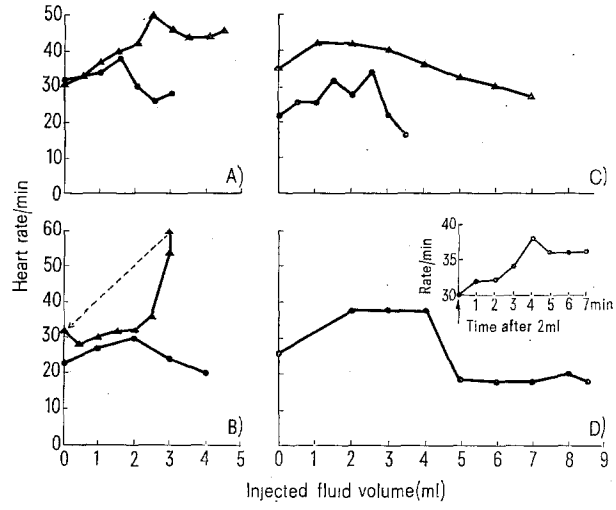

Fig. 1. 7 hearts showing initial acceleration followed by deceleration with increasing fluid volume in the pericardium. A, B and C, 2 hearts each. B, dotted line with arrow shows return of heart rate to control value after cutting open the pericardium. D, heart rate reached peak value when 2 ml Ringer was injected in first shot. Inset shows changes in heart rate with time; the peak was reached in 4 min.

Chronotropic changes with pericardial distension

Heart No.	Control heart rate/min	Maximum/ minimum heart rate/ min with distension	Distending fluid volume (ml)	Change in heart rate (%)
1	21	17	3.5	− 19
2	31	50	2.5	+ 61
3	32	38	1.5	+ 18
4	32	60	3	+ 87
5	25	62	3	+ 148
6	35	42	1	+ 20
7	26	38	3	+ 46
8	36	21	5	− 42
9	16	22	8	+ 36
10	42	30	4	− 28
11	32	22	3	− 31
12	24	32	4	+ 33
13	20	36	1	+ 80
14	24	30	2	+ 25
15	46	28	5	− 39
16	36	25	4	− 30
17	30	20	4	− 33
18	28	24	4	− 14
19	66	52	1	− 21
20	36	30	2	− 17
21	30	38	4	+ 26
22	30	24	4	− 20
23	30	20	3	− 33
24	22	34	2.5	+ 58
25	24	14	4.5	− 41
26	20	16	5	− 16

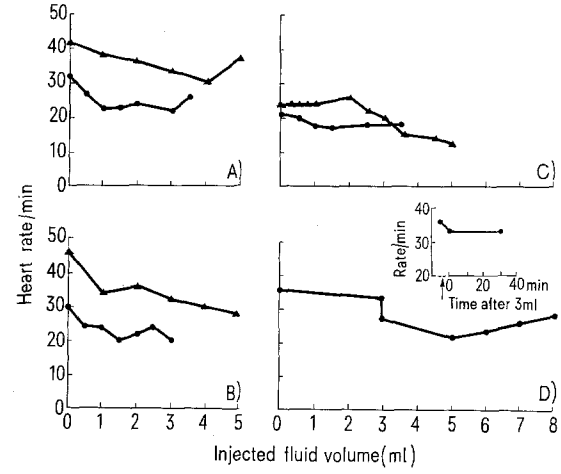

Fig. 2. Another set of 7 hearts showing progressive deceleration at increasing fluid volumes. A, B and C, 2 hearts each. D, in this heart 3 ml was injected in first shot. Inset shows changes in heart rate with time. In first 3 min the heart rate declined from 36 to 33 where it stabilized for next 30 min.

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(Figure 3). At small distending fluid volumes (1 to 4 ml), the sino-atrial conduction increased while at high distending volumes (5 to 8 ml) atrioventricular complex decreased. Release of distension by cutting open the pericardium reversed the chronotropic (Figure 1 B) and electrical (Figure 3) changes, i.e. the heart rate, voltage of ventricular complex and conduction changes tended to return to control values and arrhythmia tended to disappear.

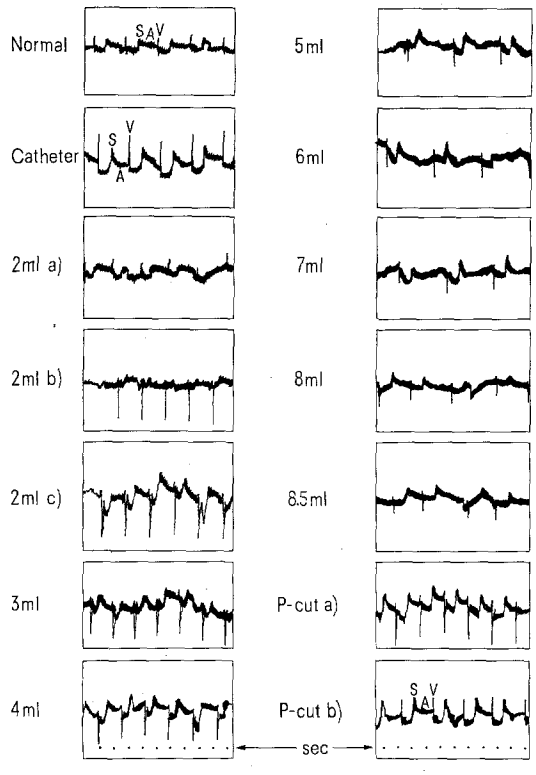

Fig. 3. Electrocardiographic changes with pericardial distension. Slight acceleration occurred when catheter was placed in the pericardium. Acceleration increased with increasing fluid volume in the pericardium upto 4 ml. Injection of more fluid caused bradycardia. S, A, V, sinus, atrial and ventricular complexes. S-A conduction increased upto 4 ml and both S-A and A-V conduction increased at 5, 6 and 7 ml. At 8 and 8.5 ml bifocal sinus discharge, arrhythmia and reduced voltage of ventricular complexes are seen. Immediately after cutting open the pericardium, rebound acceleration occurred with reversion of conduction changes and arrhythmia (P-cut, a). The rate and direction of ventricular complex reverted towards controls 10 min afterwards (P-cut, b).

The chronotropic changes in heart rate and impulse parameters due to distension were also reversed by sucking the fluid out of the pericardial sac into the syringe. Injection of air into the pericardial cavity produced changes similar to those observed by injecting Ringer's solution, and these changes were reversed by suction of air back into the syringe. The pericardium usually gave way or ruptured when 6 to 8 ml of fluid had been injected. On an average, with graded increase in fluid volume upto 5 ml the pericardial pressure rose slowly to 6–8 mm Hg. After this even small increments caused a very steep rise in the pericardial pressure to 15–25 mm Hg or more.

Discussion. The thin pericardial sac of frog heart can accommodate 6 to 8 ml of fluid. Compression of sinus venosus would alter the dimensions and configuration of pacemaking cells. It has already been shown that intraluminal pressure-stretch alters pacemaker frequency (PATHAK[1–3, 6, 7]). The present work demonstrates that extramural stretch due to pericardial distension also changes the pacemaker response. It is of interest to note that, in the hearts showing initial acceleration followed by deceleration, the pattern of chronotropic responce due to pericardial distension observed during the present work, is similar to that observed previously by intraluminal distension (PATHAK[1–3, 6, 7]). These investigations support the view that mechanical stretch of pacemaker from within or from without alters the impulse frequency and determines the chronotropic response of the heart. Moderate distension of pericardium is associated with cardio-acceleration. Relaxation of pacemaker due to compression and altered anatomy of other chambers and/or overstretch of the pacemaker appear to be responsible for bradycardia, conduction changes, atrioventricular block and arrhythmia observed at high distending fluid volumes.

Résumé. On a démontré que la distension péricardiale produit des changements chronotropiques dans le cœur de grenouille isolé en perfusion. Sur 26 cœurs, l'accélération cardiaque (18 a 148%) se produit dans 12 avec un volume de fluide péricardique atteignant 4 ml. Dans les 14 cœurs restant nous avons observé une bradycardie, des irrégularities de conduction, un blocage cardiaque et une arythmie. On en conclut que ces effects chronotropiques et les variations du paramètre d'impulsion sont dues aux changements dans l'activité du «pacemaker».

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The Effect of the Gastrointestinal Hormones on Small Intestinal Motility and Blood Flow

Recent studies give evidence that gastrin, cholecystokinin and secretin might be involved in the regulation of intestinal motility and blood flow, both in animals and in man[1–3]. Whether these effects are brought about by a direct influence on the smooth intestinal and vascular muscles, or might largely be secondary to the release of intermediary substances, is still, however, a matter of dispute. The aim of the present investigation was therefore to study in more detail the effect of graded intra-arterial infusion of the gastrointestinal hormones on the small intestinal motility and blood flow.

Methods. Experiments were performed on anaestetized cats weighing 2.5–5.2 kg and fasted for 24 h. A femoral artery was connected to a mercury manometer for recording of arterial blood pressure. The abdomen was opened along the midline and the greater omentum and the spleen were extirpated. A small intestinal segment weighing 10–20 g was isolated and the remaining parts of the intestine were extirpated. The adrenals were excluded from the circulation by encircling ligatures. The splanchnic nerves were cut just beneath the diaphragm.

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