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# DEVELOPMENTAL CHANGES IN SUBTHRESHOLD PACE-MAKER CURRENTS IN CHICK EMBRYONIC HEART CELLS

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## SUMMARY

1. Small re-aggregates of cells dissociated from the ventricles of 12-or 17-day-old chick embryonic hearts have beating properties in low potassium tissue culture medium which differ from re-aggregates prepared from 7-day-old hearts; 12-day preparations either beat with a slower rate than comparable-size 7-day preparations or they are quiescent; 17-day preparations usually do not beat.

2. A two-micro-electrode voltage-clamp technique was used to analyse the membrane currents in these preparations in the −120 to −40 mV potential range.

3. A potassium ion membrane current was measured in 12-day and 17-day preparations which was qualitatively similar to the potassium ion current measured in 7-day preparations (Clay & Shrier, 1981). It differed in that its amplitude was diminished in the ratio 1:0·2:0·03 over the 7-, 12- and 17-day sequence. Furthermore, its maximum time constant was 2 sec at both 12 and 17 days, as compared to 1 sec at 7 days, and its membrane potential range of activation was −80 to −70 mV at 12 and 17 days, as compared to −90 to −60 mV at 7 days.

4. The background current also changed with development, becoming less inward over the 7-, 12- and 17-day sequence. The voltage dependence of this change suggested that it was caused by a developmental decrease of the background sodium current.

5. The change in the background component is primarily responsible for the loss of automaticity over the 7-, 12-, and 17-day sequence.

## INTRODUCTION

In the previous paper (Clay & Shrier, 1981) we showed that aggregates of heart cells prepared from the ventricles of 7-day chick embryos contain a time-dependent current which is carried by potassium ions, and a time-independent (background) current, which may be carried in part by potassium and sodium ions. In this paper we report measurements of these currents in aggregates of ventricular heart cells prepared from embryos which were incubated either for 12 or 17 days. These older preparations exhibit changes in both current components; the time-dependent

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component is markedly diminished and the background current is less inward. The voltage dependence of the latter change is consistent with a diminished background sodium current $I_{\mathrm{Na,b}}$. These results demonstrate that developmental changes occur in pace-maker currents in the embryo during the last two weeks of incubation.

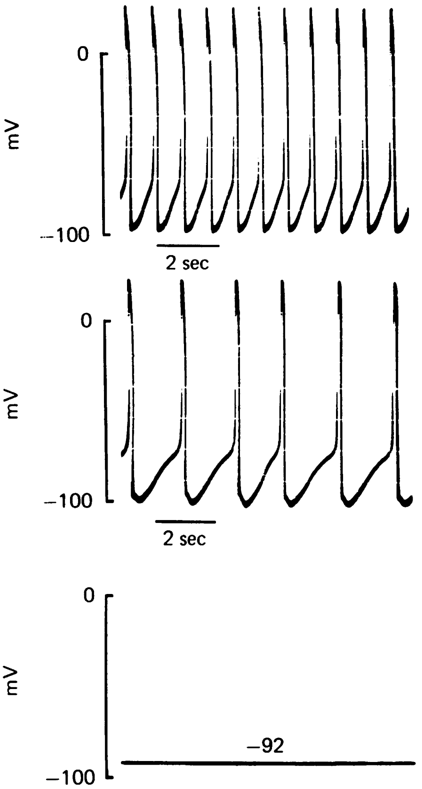

Fig. 1. Developmental changes of electrical activity at 7, 12 and 17 days ($D = 200\ \mu\mathrm{m}$, $\mathrm{K_o} = 1{\cdot}3$ mM).

## METHODS

Fertilized eggs from White Leghorn hens were incubated at 37 °C until the embryo reached the desired stage of development (12 or 17 days). The embryos were removed from the egg and the hearts were rapidly excised. In the case of 12-day-old hearts either the apical portion or the free right wall of the ventrical was dissected. The latter was also used in the case of 17-day embryos. The preparation of heart cell aggregates, the electrophysiological techniques, and the acquisition and analysis of data were all carried out as described by Clay & Shrier (1981).

## RESULTS

The physical appearance and range of diameters of heart cell aggregates prepared from 12- or 17-day embryonic hearts were similar to aggregates prepared from 7-day-old hearts. However, their beating properties were different; 12-day preparations either beat spontaneously in an external potassium concentration ($\mathrm{K_o}$) of 1·3

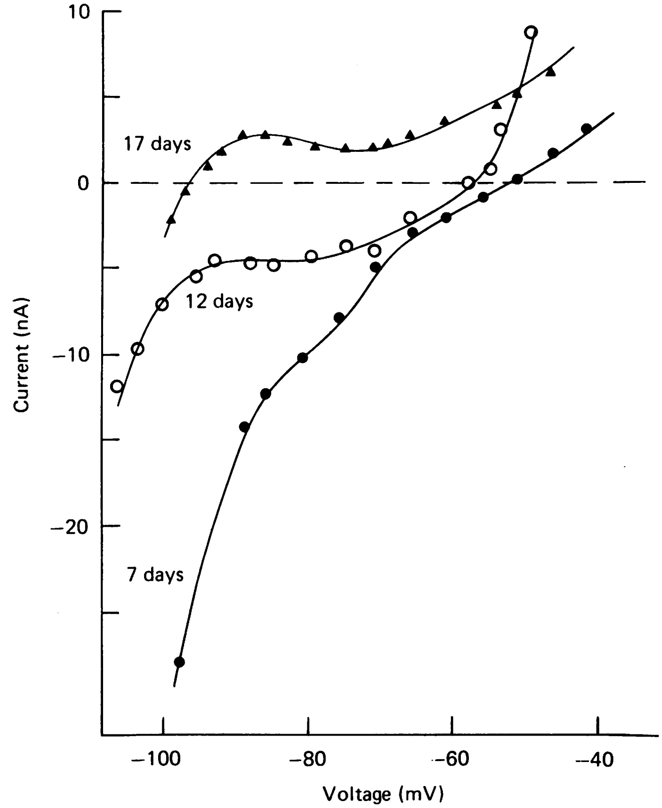

Fig. 2. Changes in *I–V* relations with development. Same preparations as in Fig. 1 with 3 $\mu$M-TTX, $K_0 = 1{\cdot}3$ mM. Lines drawn through the points by eye.

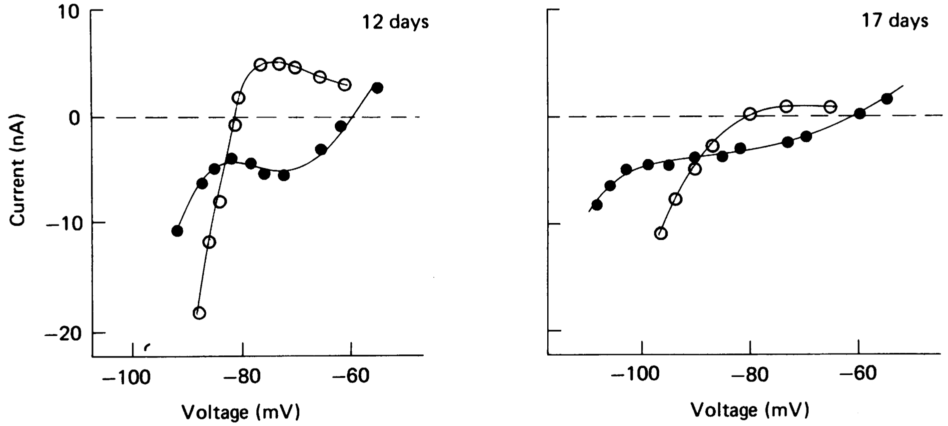

Fig. 3. Effects of elevating $K_0$ from 1·3 to 3·5 mM on *I–V* relations. The 1·3 (○) and 3·5 mM (●) curves are representative results from two different preparations at each developmental stage. Lines drawn through the points by eye.

mM or they were quiescent; 17-day preparations usually lacked automaticity. The beat rate of the spontaneous activity at 12 days was slower than the activity at 7 days, as shown by the intracellular recordings in Fig. 1. Moreover, the inter-beat interval was somewhat irregular at 12 days and a secondary hyperpolarization often occurred after the repolarization phase of the action potential (Fig. 1). All spontaneous activity in 12-day aggregates was blocked by 3 $\mu$M-TTX. Continuous trains of action potentials could be induced in these preparations with steady depolarizing current a few nA in amplitude. Similarly, 17-day preparations could be induced to beat with application of steady current. The parameters of action potentials at 7, 12 and 17 days with and without TTX are given in Table 1.

TABLE 1. Action potential parameters in a $K_o$ of 1·3 mM

| | | Action potential duration (msec) | Maximum diastolic potential (mV) | Overshoot potential (mV) | $\dot{V}_{max}$ (V.sec$^{-1}$) | $n$ |
|---|---|---|---|---|---|---|
| 7 days | Without TTX | 161 ± 15* | −88 ± 5 | 28 ± 2 | 155 ± 37 | 5 |
| | +TTX | 165 ± 10 | −92 ± 3 | 33 ± 4 | 14 ± 2 | 9 |
| 12 days | Without TTX | 177 ± 18 | −89 ± 5† | 26 ± 4 | 142 ± 36 | 5 |
| | +TTX | 159 ± 20 | −90 ± 2 | 26 ± 3 | 7 ± 2 | 5 |
| 17 days | Without TTx | 128 ± 15 | −92 ± 2 | 30 ± 5 | 176 ± 28 | 3 |
| | +TTX | 117 ± 16 | −94 ± 3 | 27 ± 4 | 9 ± 2 | 3 |

* ±S.D. of an observation.

† Maximum diastolic potential of action potentials such as those in Fig. 1 (middle panel) is taken from the faster repolarization phase of the action potential.

The developmental changes of the current–voltage ($I$–$V$) relation in $K_o$ = 1·3 mM are shown in Fig. 2; the current was less inward and its voltage dependence had an N-shaped appearance in the older preparations. The $I$–$V$ crossed the voltage axis at three points in some 12-day aggregates; these preparations exhibited two levels of resting potential, one near −60 mV, the other near −90 mV (Shrier, Clay & DeHaan, 1979). The effects of elevating $K_o$ to 3·5 mM in both 12- and 17-day preparations are shown in Fig. 3. As with 7-day aggregates, the $I$–$V$ relation was shifted in the outward direction, and it 'crossed over' the relation at $K_o$ = 1·3 mM.

The time-dependent current in response to 10 sec voltage-clamp steps is shown in Fig. 4 for a 12-day preparation ($K_o$ = 3·5 mM; holding potential, $V_H$ = −70 mV). As with 7-day aggregates, the current kinetics were described by a single exponential function of time with a voltage-dependent time constant. Moreover, potassium ions appeared to be the current-carrying species, as shown by the measurement of the equilibrium potential in Fig. 5 from a different 12-day preparation, also in $K_o$ = 3·5 mM. The equilibrium potential ($E_{eq}$) was −96 ± 3 mV ($n$ = 4) under these conditions, which corresponded to an internal potassium concentration ($K_i$) of 132 ± 15 mM. This result compared favourably with $K_i$ = 130 mM in 11-day whole hearts measured by McDonald & DeHaan (1973), although the intracellular ion activity may be less than 130 mM.

The primary difference in the current between 7 and 12 days was in its amplitude; it was markedly smaller in the older preparations, as can be seen by comparing the records in Figs. 4 and 5 with those in Figs. 3 and 6 of the preceding paper. This trend apparently continued with development after day 12, as shown by the records from a 17-day aggregate in Fig. 6 ($K_0$ = 3·5 mM); the current was barely discernible at this stage. The observation of a developmental change is quantified in Fig. 7, in which

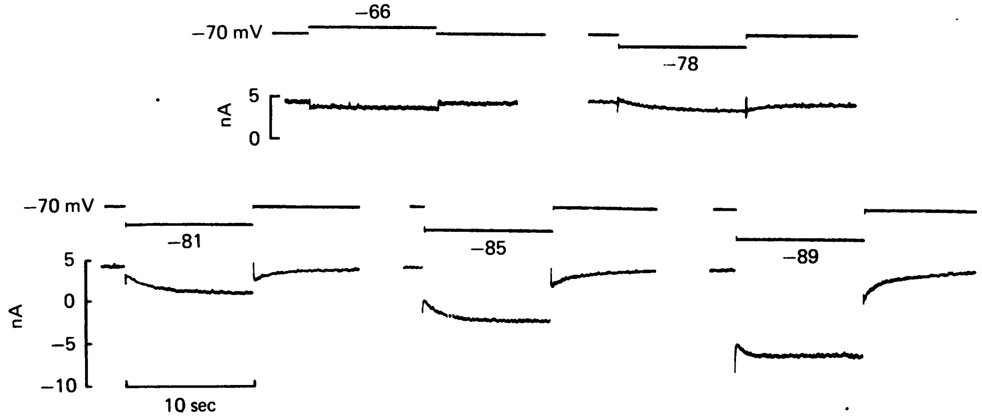

Fig. 4. Time-dependent current in response to 10 sec voltage-clamp steps in a 12-day preparation with $K_0$ = 3·5 mM. $V_H$ = −70 mV. Step potentials are as indicated. Current records filtered (low pass filter; 3 db roll-off at 50 Hz).

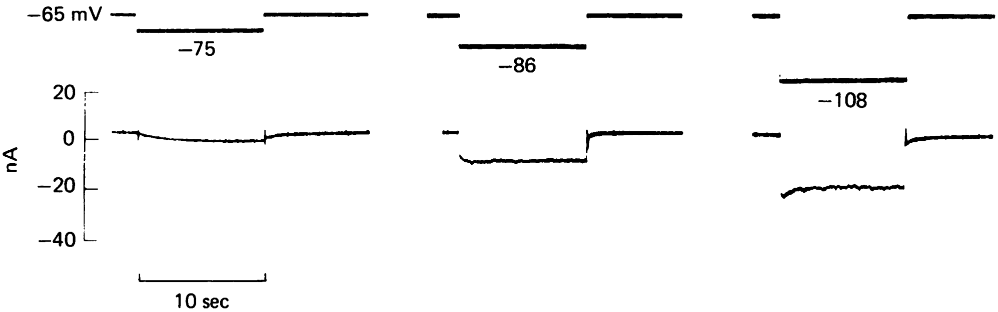

Fig. 5. Measurement of equilibrium potential in a 12-day preparation in $K_0$ = 3·5 mM (10 sec voltage-clamp steps; $V_H$ and $V_{step}$ are as indicated). Currents are filtered (low pass; 3db roll-off at 30 Hz).

the amplitudes of the time-dependent currents that occurred when the voltage was stepped back to $V_H$ ($I_b$ as in Fig. 3 of the preceding paper) are shown at 12 and 17 days, along with the theoretical representation of this current at 7 days. These results, shown on an expanded scale in Fig. 8, indicated that the voltage range of steady-state activation in both of the later stages is only about 10 mV (−80 to −70 mV), as compared to 30 mV (−90 to −60 mV) at 7 days. The theoretical lines through the data in Fig. 8 are best fits of the function $s_\infty = \alpha/(\alpha+\beta)$, where $\alpha$ and $\beta$ are the same form as eqns. (3) and (4) in the preceding paper, although the parameters $\alpha_0$, $\beta_0$, $\alpha_1$, $\beta_1$ and $V_1$ were different (Table 2).

The time constants, $\tau_s$, of the current at 12 days were similar to those at 7 days

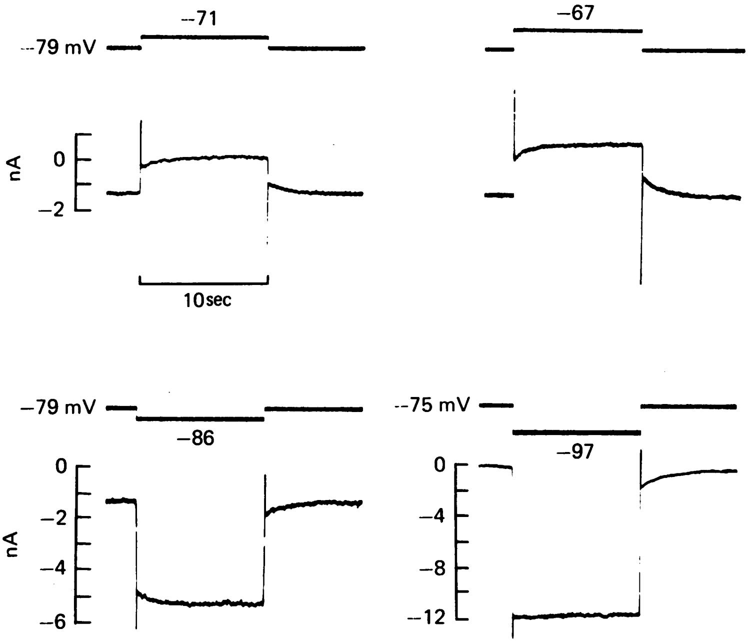

Fig. 6. Time-dependent current in a 17-day preparation with $K_0 = 3{\cdot}5$ mM (10 sec voltage-clamp steps; $V_H$ and $V_{step}$ are as indicated). Currents are filtered (low pass; 3db roll-off at 50 Hz).

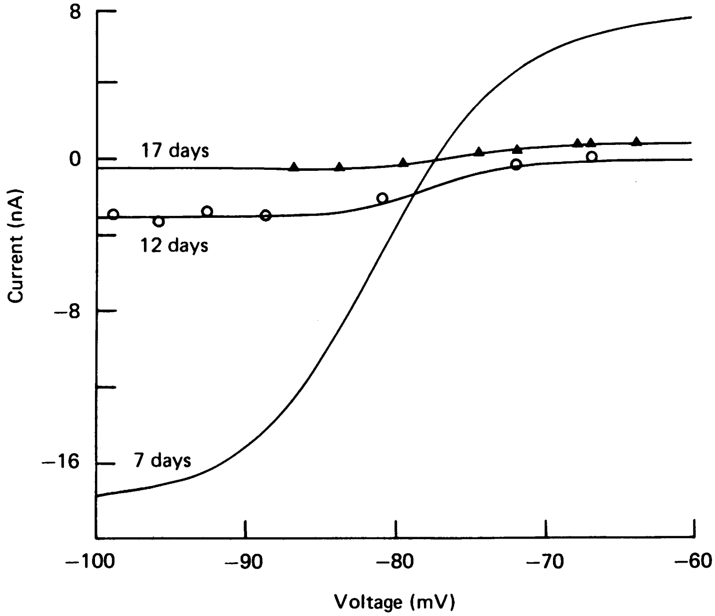

Fig. 7. Amplitude of $I_b$ currents (described in text) for a 12-day preparation (○) in $K_0 = 2{\cdot}5$ mM and a 17-day preparation (▲) in $K_0 = 3{\cdot}5$ mM. Theoretical representation of 7-day $I_b$ currents taken from Fig. 5*B* of preceding paper.

in that they did not appear to depend upon $K_0$ (Fig. 9*A*). They differed in that the peak $\tau_s$ was about 2 sec at 12 days as compared to 1·0–1·2 sec at 7 days. Moreover, the width of the bell-shaped $\tau_s$ curve was narrower, which was consistent with the $I_b$ current measurements in Fig. 8*A*. The time constants did not appear to change after day 12, as shown by the results from the 17-day preparation in Fig. 9*B*.

TABLE 2. Properties of $I_{K_2}$ current in aggregates at 12 and 17 days

| Preparation | $K_0$ (mM) | Voltage at which maximum time constant occurred† (mV) | ($I_p$) Maximum outward current‡ (nA) | Voltage at which $I_p$ occurred‡ (mV) |
|---|---|---|---|---|
| 12 days* | | | | |
| 5–11–78 | 1·3 | −64 | 7·15§ | −97·5§ |
| 5–22–78 | 1·3 | −75 | 8·10§ | −97§ |
| 5–22–78 | 2·5 | −75 | 7·05 | −88 |
| 5–22–78 | 4·8 | −75 | 8·10 | −78 |
| 1–25–79 | 3·5 | −78 | 3·60 | −85 |
| 1–26–79 | 3·5 | −79 | 6·24 | −86 |
| 4–23–79 | 1·3 | −79 | 3·80 | −117§ |
| 4–23–79 | 2·5 | −79 | 4·77 | −93 |
| 4–23–79 | 3·5 | −79 | 3·34 | −78 |
| 4–26–79 | 3·5 | −70 | 4·29 | −80 |
| 5–12–79 | 3·5 | −76 | 3·10 | −81 |
| 5–14–79 | 4·8 | −76 | 4·10 | −72 |
| 17 days* | | | | |
| 7–15–78 | 1·3 | −70 | 0·6‖ | – |
| 5–28–78 | 1·3 | −75 | 0·5‖ | – |
| 5–01–79 | 1·3 | −77 | 0·70‖ | – |
| 5–01–79 | 3·5 | −77 | 1·25 | −79 |
| 5–02–79 | 1·3 | −75 | 0·60‖ | – |
| 5–02–79 | 3·5 | −75 | 0·95 | −80 |

* Diameter of aggregates was 180–220 μm.

† As determined from the fit to the time constant data of $\tau_s = (\alpha+\beta)^{-1}$, where $\alpha = \alpha_0(V-V_1)/((1-\exp(-\alpha_1(V-V_1)))$ and $\beta = \beta_0 \exp(-\beta_1(V-V_1))$. The best-fit representation of all the data corresponded to $\alpha_0 = 1{\cdot}65\ \mathrm{sec}^{-1}$, $\alpha_1 = 0{\cdot}28\ \mathrm{mV}^{-1}$, $\beta_0 = 0{\cdot}020\ \mathrm{sec}^{-1}$, $\beta_1 = 0{\cdot}15\ \mathrm{mV}^{-1}$ in these theoretical functions. The peak time constant of $\tau_s$ is 2·05 sec, which occurs at $V = V_1 - 17$ mV. That is $V_1$ equal to the number in the third column plus 17 mV.

‡ As determined from the best fit of eqns. (1) and (2) of the preceding paper to the fully activated *I–V* relation with $r = 2$ and $\beta = 0{\cdot}5$.

§ Extrapolated from the best fit of eqns. (1)–(2) of the preceding paper to the fully activated *I–V* relation.

‖ Amplitude of time-dependent current at 17 days in $K_0$ = 1·3 mM was measured directly from voltage steps in −90 to −60 mV membrane potential range.

Results of the ratio analysis of the time-dependent currents during and after voltage-clamp steps (Noble & Tsien, 1968) are shown in Fig. 10 for comparable-size 7-, 12- and 17-day preparations ($D = 200$ μm). The measurements of the peak amplitude of the current ($I_p$) from all experiments are given in Table 2. The relative dependence of the current on both $K_0$ (Table 2) and voltage (Fig. 10*B*) at 12 and 17 days was the same as at 7 days. However, the amplitude of the current was smaller

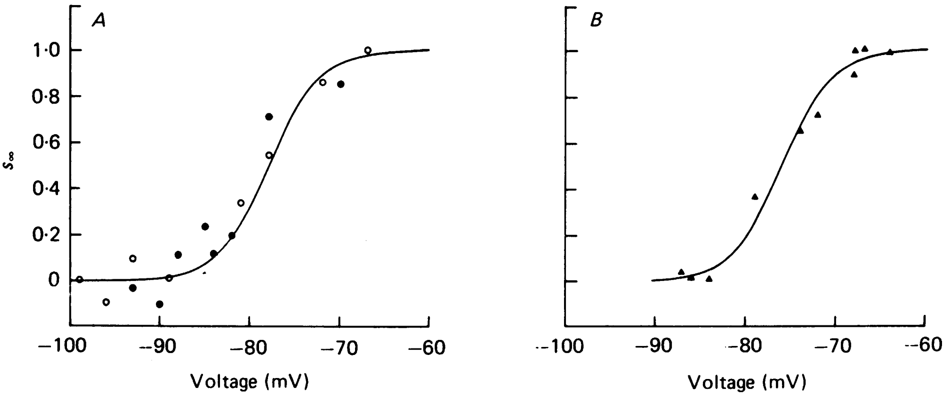

Fig. 8. Normalized $I_b$ currents. *A*, 12-day preparation in $K_0 = 2·5$ mM (○) and 3·5 mM (●). Same preparation for both cases. Theoretical line is $s_\infty = \alpha/(\alpha+\beta)$, where $\alpha$ and $\beta$ are given eqns. (3) and (4) of preceding paper with $\alpha_0 = 1·65\ \text{sec}^{-1}$, $\beta_0 = 0·020\ \text{mV}^{-1}$, $\alpha_1 = 0·28\ \text{sec}^{-1}$, $\beta_1 = 0·15\ \text{mV}^{-1}$ and $V_1 = -61$ mV. *B*, 17-day preparation in $K_0 = 3·5$ mM. Same preparation as in Fig. 7. Theoretical line is same as in *A* with a 2 mV shift in the depolarizing direction.

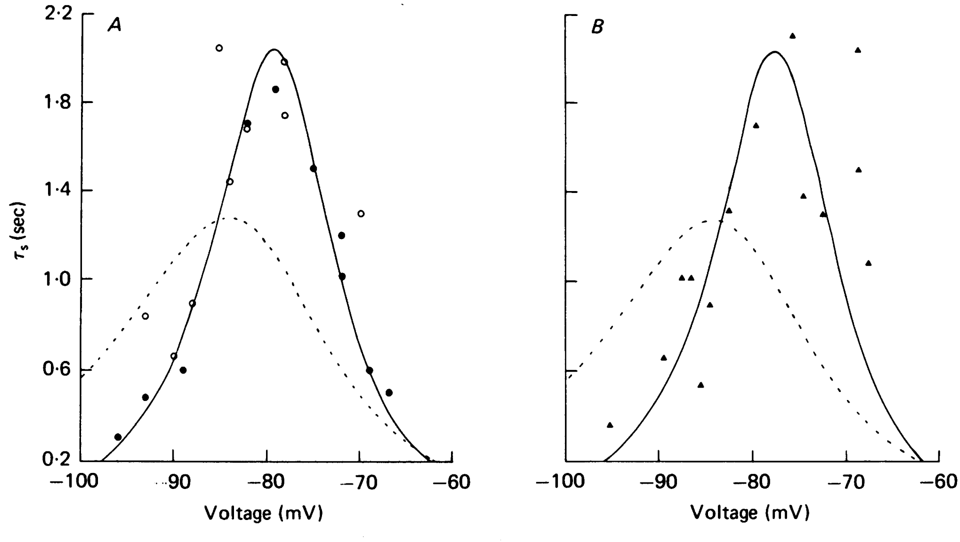

Fig. 9*A*. Time constants for a 12-day preparation in 2·5 mM $K_0$ (○) and 3·5 mM $K_0$ (●). Same preparation as in Fig. 8*A*. *B* time constants for a 17-day preparation (▲). Same preparation as in Fig. 8*B*. Solid lines are $\tau_s = (\alpha+\beta)^{-1}$, where $\alpha$ and $\beta$ are the same as in Fig. 8*A* and 8*B*. Dashed line is a theoretical representation of 7-day time constants taken from Fig. 5*A* of preceding paper.

in the older preparations, as shown in Fig. 10*A*. The lines through the data represent the model of inwardly rectifying membrane current given in eqns. (1) and (2) of the preceding paper with $r = 2$, $\beta = 0·5$ and $N_s e/t$ chosen to give the best fit to the 7-, 12- and 17-day results, respectively.

The background current, $I_{bg}$, was also different in the older preparations, as shown directly by the $I$–$V$ relations in $K_0 = 1·3$ mM in Fig. 2. The time-dependent component was virtually absent for $V \lesssim -90$ mV, and was less than 1 nA at all potentials in $K_0 = 1·3$ mM both in 12- and 17-day aggregates. That is, the $I$–$V$ relations at 12 and 17 days in Fig. 2 are essentially direct measurements of $I_{bg}$ for $V < -55$ mV. The difference between these curves describes the developmental change in $I_{bg}$ between 12 and 17 days. We have modelled these results with the three-current description (preceding paper) of $I_{bg}$ (Fig. 11). We first applied the model to the $I$–$V$ relation at 7 days by subtracting the time-dependent component as in the preceding paper. This result was fitted with the three-current model (lower continuous line in Fig. 11). We found that the $I$–$V$ relations at 12 and 17 days in $K_0 = 1·3$ mM were also adequately described by the same parameter fit, if the background sodium conductance $G_{Na,b}$ was changed from $8·5 \times 10^{-3}$ mS.cm$^{-2}$ at 7 days to $4·7 \times 10^{-3}$ mS.cm$^{-2}$ at 12 days, and to $2·5 \times 10^{-3}$ mS.cm$^{-2}$ at 17 days; and if the reversal potential of the inward rectifying component of the model was changed from $-95$ mV at 7 days to $-108$ mV at 12 days, and to $-102$ mV at 17 days. The upper two continuous lines in Fig. 11 represent the model with these parameter changes. Results of curve fitting of all the $I$–$V$ relations at 12 and 17 days in $K_0 = 1·3$ mM are shown in Table 3. The principal conclusion of this analysis is that the primary change in $I_{bg}$ over the 7-, 12- and 17-day sequence is a loss of inward sodium current in the pace-maker voltage range.

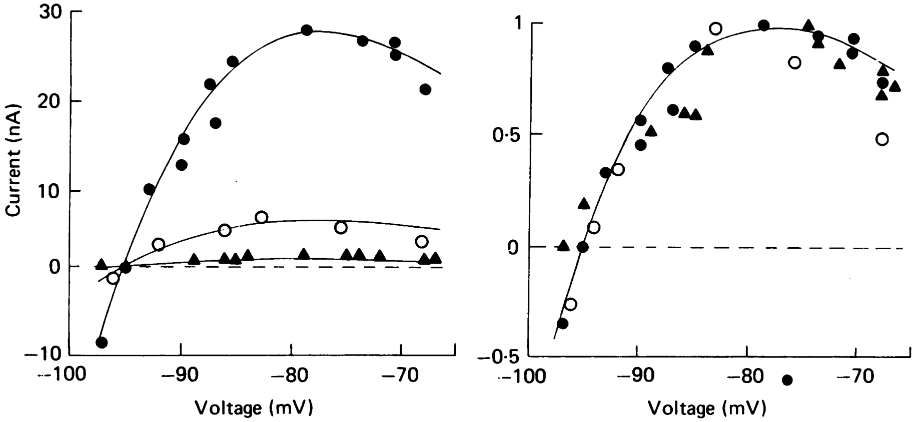

Fig. 10*A*. Open-channel $I$–$V$ relations of $i_{K_2}$ in $K_0 = 3·5$ mM from 7- (●), 12- (○) and 17-day (▲) preparations of comparable size ($D = 200\,\mu$m) obtained from the ratio analysis (Noble & Tsien, 1968). The 12-day curve was arbitrarily shifted 2 mV in the depolarizing direction to enhance the comparison. Continuous lines are theoretical $I$–$V$ relations from eqns. (1) and (2) of the preceding paper with $r = 2$, $\beta = 0·5$ and $Ne_s/t = 1·09\,\mu$A for 7 days, 0·14 $\mu$A for 12 days and 0·028 $\mu$A for 17 days. *B*, relative voltage dependence of the 7-, 12- and 17-day open-channel $I$–$V$ relations obtained by normalizing each set of data in *A* by corresponding peak outward current ($I_p$).

The time-dependent $I_x$ current described in the preceding paper was only a few nA in amplitude in the $-55$ to $-40$ mV range in $K_0 = 1·3$ mM in 7- and 17-day aggregates. However, it was relatively significant in low $K_0$ in 12-day preparations.

This current accounts for the difference between the data points of the $I$–$V$ relation at 12 days in Fig. 11 and the corresponding $I_{bg}$ model for $V \gtrsim -55$ mV. That is, the $I_x$ current appeared to be greater in 12-day than in either 7- or 17-day preparations.

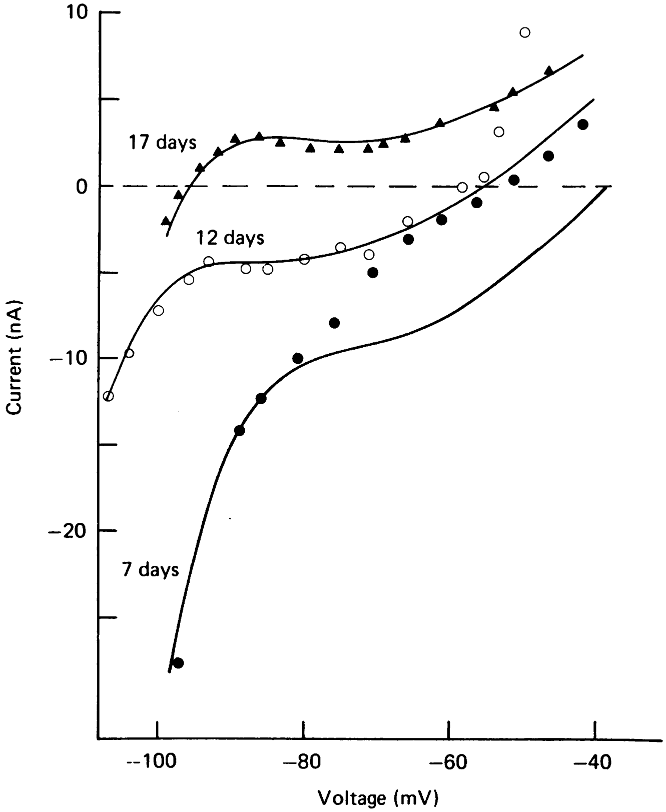

Fig. 11. Model representation of $I_{bg}$ current. Data represents the same $I$–$V$ relations as in Fig. 2. Continuous lines represent the three-current model of $I_{bg}$ described in the text and the preceding paper;

$$I_{bg} = G_{K_4}(V - V_4)/\ (1 - \exp(-k_B T(V - V_4)/e)) + G_{Na,b}(V - EN_a) + I_{K_3},$$

where $I_{K_3}$ is the $r = 4$ form of eqns. (1) and (2) of the preceding paper, with $\beta = 0{\cdot}63$, $Ne/t = 14{\cdot}1\ \mu A . cm^{-2}$, $G_{K_4} = 0{\cdot}024\ mS . cm^{-2}$, $V_4 = -40$ mV and $E_{Na} = +40$ mV: For the 7-day curve $G_{Na,b} = 0{\cdot}0094\ mS . cm^{-2}$, $E_{eq}$ (equilibrium potential for $I_{K_3}$) = −95 mV; for the 12-day curve, $G_{Na,b} = 0{\cdot}0051\ mS . cm^{-2}$, $E_{eq} = -108$ mV; for the 17-day curve $G_{Na,b} = 0{\cdot}0028\ mS . cm^{-2}$, $E_{eq} = -102$ mV. The difference between the data and the curve at 7 days is the steady-state amplitude of the $I_{K_2}$ component, which is only about 1–2 nA at 12 days and even smaller at 17 days. Consequently, the $I$–$V$ relations of these latter stages in $K_o = 1{\cdot}3$ mM is practically the same as $I_{bg}$ for $V \lesssim -55$ mV. The deviation between the 12-day $I$–$V$ relation and the $I_{bg}$ model for $V \gtrsim -55$ mV represents the steady-state value of the $I_x$ component, as described in the text.

## DISCUSSION

### *Membrane currents and action potential parameters*

We have described three currents in heart cell aggregates: a time-dependent potassium pace-maker current, $I_{K_2}$; a time-independent pace-maker current, $I_{bg}$, which is carried by sodium, potassium and possibly other ions; and a second time-dependent current with slow kinetics, $I_x$, which appears to be involved with the repolarization phase of the action potential and acts as a barrier to beating in TTX-treated aggregates. The TTX-sensitive sodium current is associated with the upstroke velocity of the action potential, and appears to be fully developed prior to day 7 (Nathan & DeHaan, 1979). Our measurements of $\dot{V}_{max}$ (Table 1) support this conclusion. However, the $\dot{V}_{max}$ parameter differs over the 7-, 12- and 17-day sequence in TTX-treated preparations, changing from 14 V/sec at 7 days to 7–9 V/sec at 12 and 17 days. The difference in these rather low values of $\dot{V}_{max}$ could be attributed either to differences in the rate of pace-making, or to differences in the $I_x$ current. The latter component is larger at 12 days as compared to 7 days which might explain the lower $\dot{V}_{max}$ in TTX at this stage. The only other statistically significant developmental change in the action potential parameters (Table 1) is in the duration, which is 160–170 msec at 7 and 12 days compared to 120–130 msec at 17 days. A developmental change in the $I_x$ current kinetics is one possible mechanism underlying this change. However, we cannot rule out other mechanisms, since we have not yet carried out a complete investigation of the $I_x$ current or the slow inward current ($I_{si}$).

TABLE 3. Analysis of $I_{bg}$ in $K_o$ = 1·3 mM with three-current model*

| | | $I_{K_3}$ | |
|---|---|---|---|
| Preparation | $G_{Na,b}$ (mS.cm$^{-2}$) | $Ne/\bar{t}$ ($\mu$A.cm$^{-2}$) | $E_{eq}$ (mV) |
| 7 days | | | |
| 2-20-78 | 0·0094 | 14·1 | −95 |
| 5-12-78 | 0·0094 | 21·5 | −95 |
| 5-14-79 | 0·0117 | 22·4 | −90 |
| 12 days | | | |
| 5-08-78 | 0·0028 | 21·5 | −90 |
| 5-11-78 | 0·0070 | 14·1 | −95 |
| 5-22-78 | 0·0044 | 21·5 | −100 |
| 4-10-79 | 0·0051 | 14·1 | −108 |
| 4-23-79 | 0·0051 | 11·2 | −100 |
| 17days | | | |
| 7-15-79 | 0·0028 | 14·1 | −102 |
| 5-30-79 | 0·0042 | 14·1 | −100 |
| 5-01-79 | 0·0037 | 18·8 | −108 |
| 5-02-79 | 0·0042 | 11·7 | −90 |

* Parameters of $I_{K_4}$ are $G_{K_4}$ = 0·024 mS.cm$^{-2}$, $V_4$ = −40 mV, in all cases except 7-day 5-14-79, for which $G_{K_4}$ = 0·048 mS.cm$^{-2}$ and $V$ = −50 mV. Conductances were scaled by $2·14 \times 10^{-2}$ cm$^2$, which is the calculated membrane surface area for $D$ = 200 $\mu$m.

The measurement of maximum diastolic potential at 7 days (Table 1) might suggest an effect of TTX on the background sodium current at this stage. However, the statistical overlap of these results indicates that the difference is not significant.

### *Developmental changes in membrane currents*

The measurements of $I_{K_2}$ in the 7-, 12- and 17-day sequence provide our most complete description of developmental change of membrane current in aggregates. This component is carried by potassium ions at all three stages, but both its amplitude and kinetics change. Since aggregates are a mixed population of myocytes and non-cardiac cells, one possible mechanism for the loss in current amplitude might simply be an increase in the fraction of non-cardiac cells from 20 % at 7 days (Sachs & DeHaan, 1973) to higher levels in the older preparations. However, some of our experiments on 12- and 17-day preparations were carried out on enriched cultures in which the fraction of non-cardiac cells was smaller than 20 %. The results from these preparations were no different than those from non-enriched preparations. Consequently, we have interpreted our results in terms of changes in membrane currents rather than a change in number of myocytes.

A reduction in a specific membrane current could be caused either by a loss of ionic channels per unit area, or by a reduction of the single-channel conductance (cf. DeFelice, 1977). Measurements of membrane current noise from the 7-, 12- and 17-day sequence could, in principle, distinguish between these two mechanisms (DeFelice, 1977).

The time constants of $I_{K_2}$ are modified between 7 days and 12 days; they appear to change no further between 12 days and 17 days, although the small amplitudes of the current at the latter stage preclude precise measurements of $\tau_s$. This change in the $I_{K_2}$ gating kinetics is difficult to interpret, because the molecular mechanisms underlying the channel gating process in biological membranes are poorly understood.

The change in $I_{bg}$ may, in principle, be attributed to either an inward or an outward current. The voltage-dependent shape of $I_{bg}$ at the different developmental stages gives a clue as to which change may be dominant. The position of $I_{bg}$ on the current and voltage axes, its inward rectification, and its dependence on $K_0$ all suggest that it is carried in part by potassium ions. The inward rectifying component must also be carried in part by an ion having a reversal potential positive to −95 mV at 7 days in order to explain the deviation of its equilibrium potential from $E_K$. Since the shape of the inward rectification remains approximately the same over the 7–, 12– and 17-day sequence (Fig. 11), we conclude that this part of $I_{bg}$ is not greatly modified by development. We attribute the changes in $I_{bg}$ to the background sodium current, $I_{Na,b}$. The available evidence suggests that $I_{Na,b}$ is a linear function of voltage (McAllister, Noble & Tsien, 1975; Beeler & Reuter, 1977). If we assume that sodium current in chick ventricle has a similar voltage dependence, we find that the changes in $I_{bg}$ can be nicely described by a reduction of $G_{Na,b}$ from about 0·010 mS . cm$^{-2}$ at 7 days to 0·003 mS . cm$^{-2}$ at 17 days. It is interesting to note that the latter value is the same as that used by Beeler & Reuter (1977) for $G_{Na,b}$, in their model of the action potential of adult ventricular muscle. These results should be viewed with caution, however, since the curve fitting procedure provides only an indirect indication of changes in $I_{bg}$; sodium tracer flux measurements, preferably from aggregates, will be required to test our prediction concerning $G_{Na,b}$.

### *Significance of results for whole embryonic heart*

As mentioned in the previous paper, electrophysiological measurements from whole heart are difficult to interpret in terms of membrane ionic currents because of voltage and potassium ion concentration inhomogeneities in the cleft spaces. With this proviso in mind, some tentative comparisons may be made betwen our measurements and those of $E_R$ and potassium fluxes from whole embryonic chick hearts. Carmeliet, Horres, Lieberman & Vereecke (1976) found that 19-day hearts rested at $-68$ mV with $K_0 = 0$, and at $-85$ mV with $K_0 = 2{\cdot}5$ mM. Our results (Fig. 3) indicate that the dependence of $E_R$ on $K_0$ at this developmental stage is caused by the influence of $K_0$ on $I_{K_3}$. That is, the U-shaped dependence of $E_R$ on $K_0$ which Carmeliet *et al.* (1976) observed in 7- and 19-day hearts is attributable to a different mechanism at each stage: $I_{K_2}$ at 7 days (preceding paper) and $I_{K_3}$ at 19 days. The $E_R$ of older hearts was consistently negative to $E_R$ at 7 days (Carmeliet *et al.* (1976), which we attribute to a developmental loss of $I_{Na,b}$.

Carmeliet *et al.* (1976) also found that the resting potassium efflux in 19-day hearts was 65–70 % greater than it was in 7-day hearts for $K_0 = 0$ and 2·5 mM. This difference was 15–30 % for higher levels of $K_0$. These results would appear to conflict with our observation of a developmental loss of $I_{K_2}$ potassium current. However, the older hearts rest at a more negative $E_R$. Since the $I_{K_3}$ current increases with hyperpolarization, this component and the steep voltage dependence of one-way flux through a multi-site single-file channel (Hodgkin & Keynes, 1955) can account for the increase in potassium efflux between 7 and 19 days, even though $I_{K_2}$ is virtually nil in older hearts.

### *Relationship of developmental changes to beating*

The changes in the $I$–$V$ relations over the 7-, 12- and 17-day sequence are unambiguously related to the changes in beating shown in Fig. 1. The $I$–$V$ relations of quiescent 12-and 17-day preparations cross the voltage axis in the $-100$ to $-90$ mV region. The primary developmental change in current in this potential range is in $I_{bg}$, which becomes less inward with development; we have attributed this result to a developmental loss of $I_{Na,b}$. The relationship of the loss of $I_{K_2}$ to loss of beating is secondary, although it may partially account for the increase in inter-beat interval between 7 and 12 days (Fig. 1).

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