attained. As seen in the Figures 1 and 3, we always obtained in the *a*-wave 2 negative deflections at the rectal temperature between 27 and 28 °C. Each peak latency was measured from the onset of the stimulus to the trough. In the chick embryo of 18 days, the peak latency was 30–50 msec for the first deflection (a_1), and 80–120 msec for the second (a_2). In 3-day-old chicks, the latency of the first is similar to that of 18 days of incubation, but the latency of the second is shorter (45–90 msec). Two *a*-waves called a_1 and a_2 waves have been reported in mammals[8–10] and frogs[11]. The evidence from depth recording in the frog indicates that the a_1 is from the receptors while the a_2 is from the inner nuclear layer[11]. In the chick, GARCIA-AUSTT and PATETTA-QUEIROLO[2] have already mentioned that the *a*-wave was often broad and quadrangular with a notch in its midpoint on the chick embryo of 18 days. Further evidence is provided by WITKOVSKY[4] who demonstrated that cornea-negative activity could be divided into 2 components, a_1 and a_2 waves, in the chick embryo. It is of interest that the division between a_1 and a_2 waves could be accentuated by repetitive light stimulation[4]. Two negative deflections obtained in the present experiment may be comparable to the a_1 and a_2 waves, though the further analysis remains to be investigated.

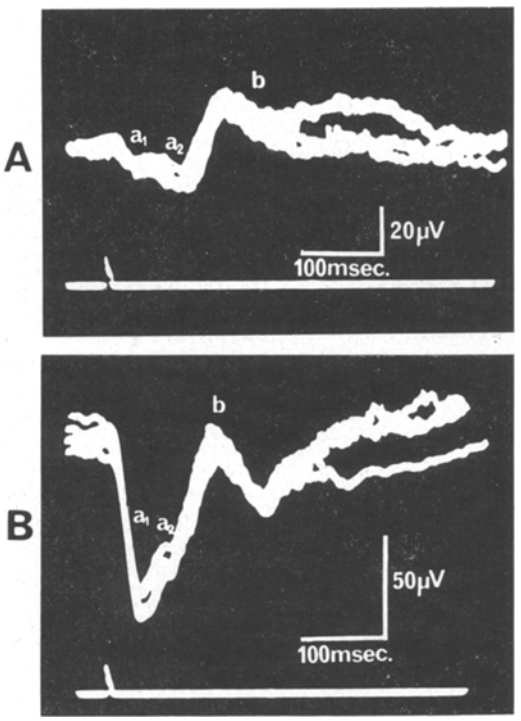

Fig. 3. Effect of decrease of body temperature on ERG in a chick embryo at 18 days of incubation (A) and in a 2-day-old (B). 4 successive sweeps have been superimposed in A and B records at the rectal temperature of 28 °C, respectively. The stimulus was given at the point marked by the bottom record in every case.

In addition, we often observed the 2 components on the *b*-wave during the course of decreased body temperature, as could be seen in Figure 1 at the rectal temperature between 25 and 26 °C. Similar double *b*-waves have been reported in the chick anesthetized with pentobarbital[4,5]. Since it has been shown in the previous[7] as well as the present study, that double *b*-wave was never observed under normal conditions, it is suggested that these might be caused under the abnormal conditions induced by a decreased body temperature or anesthesia. Changes in the pattern of the *b*-wave comparing with that in vitro will be reported in the subsequent paper[10,12].

Zusammenfassung. Untersuchung der Beziehung zwischen ERG und Körpertemperatur bei Küken ergab Abnahme der *b*-Wellen bei Verminderung der Rektaltemperatur, ohne Beeinflussung der Amplitude der *a*-Welle.

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The Inhibitory Effect of Sodium on the Contraction of Frog's Heart Perfused with Sucrose Solution

Frog's heart continues to beat spontaneously and to respond to electrical stimulation for 5–6 h if perfused with half isotonic solution of sucrose at 18–20 °C[1–7]. At higher temperatures (25–32 °C), the heart does not beat unless 0.1–0.2 m*M* sodium pyrophosphate (PP) or PP with 0.05–0.2 m*M* adenosinetriphosphate (ATP) is added to the sucrose solution[8]; addition of sodium salts of PP and ATP introduces sodium up to 1.2 m*M* in the sucrose solution. The beneficial effect of PP and ATP is not due to sodium, since addition of an equivalent quantity of sodium chloride or more (up to 2.4 m*M*) produces no such effect; the beneficial effect is therefore due to PP and ATP parts of the molecules. At still higher temperatures (37–38 °C), PP and ATP become ineffective and the heart beats for 1–2 h if sodium citrate (0.8 m*M*) is added to the sucrose solution. Addition of equivalent quantity or more (up to 3.6 m*M*) of sodium chloride produces no beneficial effect.

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In further experiments it was found that the optimum temperature for the spontaneous contractions and the response to electrical stimulation when perfused with half isotonic solution of sucrose is 19 °C (Figure 1). It makes no significant difference if the sucrose solution is deionized to remove traces of calcium (0.01–0.015 mM); correspondingly addition of calcium (0.01–0.05 mM) to the sucrose solution has no significant effect. Higher concentrations of calcium produce contracture and inhibit the mechanical response.

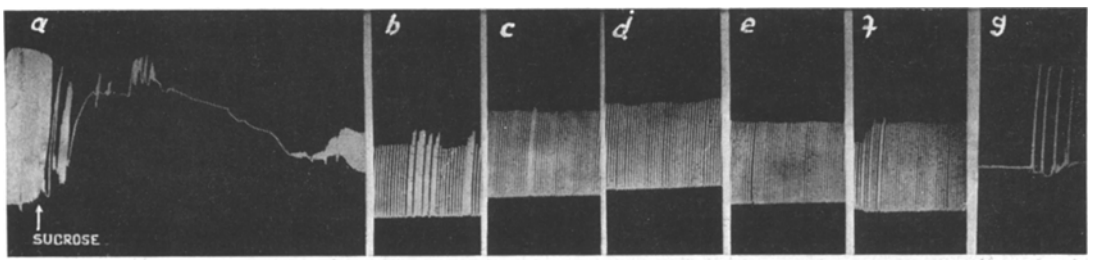

Fig. 1. Effect of perfusion of frog's heart with half isotonic (0.112 M) solution of sucrose at 19 °C. (a) Beginning of perfusion with sucrose solution. (b–g) Rhythmic contractions after perfusion with sucrose solution for 1, 2, 3, 4 and 6 h respectively.

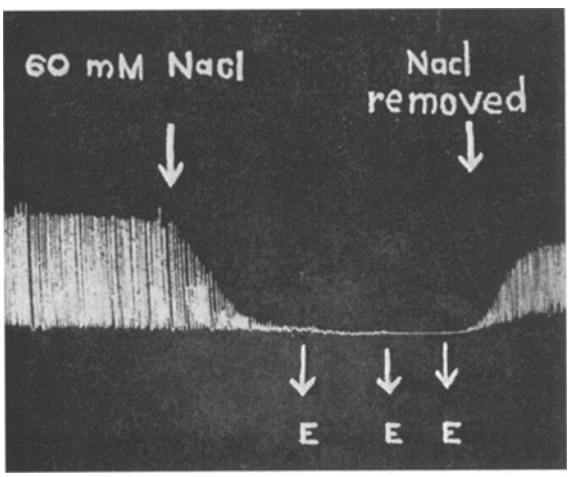

Fig. 2. The inhibitory action of sodium chloride (between arrows) on the rhythmic contractions and the response to electrical stimulation of frog's heart perfused with half isotonic solution of sucrose containing sodium pyrophosphate (0.2 mM) at 30 °C. The sucrose solution was replaced with half isotonic (60 mM) solution of sodium chloride. At (E) the heart was directly stimulated by single shocks of 5 msec duration and 20 V.

After the heart has adjusted to half isotonic solution of sucrose in $^1/_2$–1 h, replacement of the sucrose solution with 0.112 M sodium chloride[1], or Ringer solution[4], temporarily stops the heart. When perfused with sucrose solution containing PP and ATP, the action of sodium chloride (2.5–120 mM) similarly becomes inhibitory[8]. In further experiments it has been found that when the frog's heart is perfused with half isotonic solution of sucrose containing PP (0.1–0.2 mM) or PP with ATP (0.1–0.2 mM), sodium not only inhibits the spontaneous contraction, but also the response to electrical stimulation (single shocks of 5 msec duration and 5–20 V); the action of sodium can be tested by adding small quantities (10–20 mM) of sodium chloride to the sucrose solution, or the sucrose solution replaced with half isotonic (60 mM) solution of sodium chloride (Figure 2), half isotonic or isotonic Ringer solution. Addition of calcium (0.02–0.05 mM) along with the sodium makes no significant difference. Thus, in a heart which has adjusted to sucrose solution, the action of sodium is inhibitory to excitability in general.

In further experiments, it was also found that the action of sodium chloride becomes inhibitory to spontaneous contractions and the response to electrical stimulation, if the heart is perfused with half isotonic

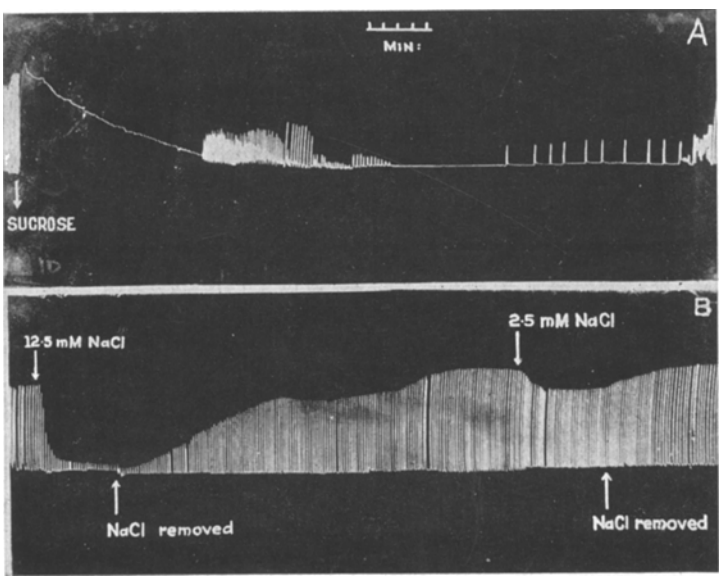

Fig. 3. The inhibitory action of sodium chloride (between arrows) on frog's heart perfused with half isotonic solution of sucrose containing sodium citrate (0.8 mM) at 37 °C. (A) Beginning of perfusion with sucrose solution. (B) Rhythmic contractions after perfusion for 1 h.

solution of sucrose containing 0.8 m*M* sodium citrate with 0.05 m*M* calcium chloride (Figure 3).

The inhibitory effect of sodium on the mechanical response suggests that sodium is not responsible for depolarization in the heart perfused with half isotonic solution of sucrose. But before the heart has adjusted to the sucrose solution, the action of sodium is stimulatory (Figure 4) and, therefore, in Ringer solution sodium is responsible for depolarization. This suggests the existence of 2 excitabilities in the frog's heart.

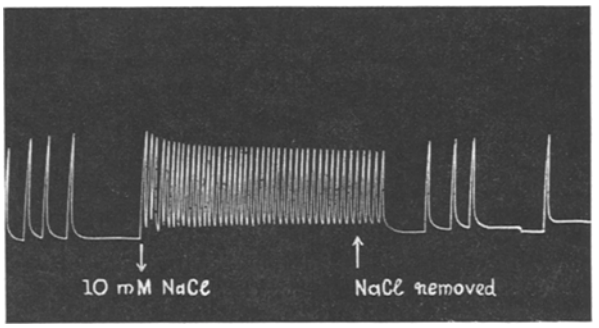

Fig. 4. The stimulatory action of sodium chloride (between arrows) on frog's heart perfused with half isotonic solution of sucrose for $^1/_2$ h.

Efflux of anions might be responsible for excitability and action potential in sucrose solution. But the effects of cation influx and anion efflux should reinforce and not antagonize each other. These experiments therefore support the suggestion that in frog's heart there are 2 independent and antagonistic mechanisms for excitability and action potential[8]. One of them is dependent upon ionic fluxes[9], and the other independent of such fluxes[5].

Résumé. Le cœur de grenouille continue à battre de lui-même et répond à une stimulation électrique durant 5–6 h s'il reçoit une injection contenant une demi solution isotonique de saccharose, de pyrophosphate de sodium et d'adénosinetriphosphate ou de citrate de sodium. Le chloride de sodium annule ces deux réactions.

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Variations of Plasma Kininogen Content due to High Sodium Intake in Rats[1]

Kininogen are plasma proteins contained in the pseudoglobulin fraction, which precipitate with ammonium sulfate between 33 and 46% saturation[2]. They are substrates to various enzymes such as trypsin[3], kallikreins[4,5] and others that liberate vasoactive peptides (Plasma kinins), when incubated in appropriate conditions.

Some physiological and pathological roles have been attributed to kinins[6–11]. Preliminary observations induced us to think of a relation between the kallikrein-kininogen-kinin system and the metabolism of sodium so far not described. The present work was done to measure the variations of the kinin substrate under conditions of sodium loading.

Materials and methods. Male albino rats (Houssay strain), weighing between 150 and 200 g were used. They were fed with standard rat food, containing about 100 mg sodium per 100 g; distilled water, 1% and 2% NaCl solutions were used as drinking fluids.

Blood (0.7 ml) was obtained by cardiopuncture (always performed between 14.00 and 15.00 h) with a heparinized syringe and immediately centrifuged at 1500 *g* at 4 °C for 30 min. Plasma was separated and kininogen samples were prepared according to FASCIOLO et al.[12]. Standard bradykinin used for bioassay was BRS640, Sandoz Lab., Basel, Switzerland.

Experimental and results. Several kinds of experiments were performed: A group of 5 rats was given distilled water ad libitum, and blood was drawn after 3 days, then water was replaced by 1% NaCl and on day 6 blood was drawn again. This procedure was repeated again, giving water for 3 days and 1% NaCl for 3 more days, and blood was drawn at the end of each period. Plasma Kininogen Content (PKC) was estimated (Figure 1). There was a significant decrease of PKC after the administration of NaCl solution as drinking fluid. The return to the water diet increased PKC in each single rat, but the values remained below the initial ones after 3 days of water intake.

Similar results were obtained in a differently designed experiment: a group of 16 rats was put in cages and given standard rat food. They drank distilled water and blood was drawn on days 0 (12 rats) and 3 (16 rats). On the third day, water was replaced by 1% NaCl and blood was drawn again on days 6 (16 rats) and 9 (13 rats, 3 died after cardiopuncture on day 6). PKC was also estimated.

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