Material and methods. In our present experiments the effects of Pi, of G and of PAH loading were studied by clearance techniques in anaesthetized dogs with one kidney denervated. Denervation was performed by left splanchnicotomy 10–70 days prior to the experiments.

GFR was measured by the clearance of ^{51}Chromium edetic acid (^{51}Cr-EDTA), except for the PAH loading series where clearance of inulin[26] was used. The concentration of Pi and PAH, in plasma and urine samples, were determined by methods of FISKE and SUBBAROW[27] and of BRATTON and MARSHALL[28], respectively, that of G was measured enzymatically (GOD-Perid, Boehringer).

Results and discussion. Tubular transport was calculated from the filtered and excreted quantities. Plasma (P) Pi, G and PAH levels (mean values and range), GFR, rate of tubular transport of innervated (inn) and denervated (den) kidneys with standard errors (S.E.), respectively, and results of statistical evaluation by Student's *t*-test are presented in the table.

Denervation phenomenon, i.e. significantly increased urine flow and sodium excretion from denervated kidneys, was present in all the experimental series with no difference in GFR between intact and splanchnicotomized side.

The results show that maximum transport (Tm) of Pi, of G and of PAH, respectively, was depressed after denervation. Considering that, in addition to diminished proximal tubular sodium reabsorption, a decrease in other mainly proximal tubular active secretory or reabsorptive transport processes without any change in GFR was observed, an impairment of proximal tubular transport function after denervation can be suggested. The mechanism of this phenomenon is not clear as yet. However, the defect of essentially different transport processes allows one to conclude that renal sympathetic activity might in general regulate proximal tubular transport functions.

Effect of inorganic phosphate, D-glucose and para-aminohippuric acid loading on their transport in denervated and innervated kidneys.

		Pi	G	PAH
m		5	9	9
n		17	15	15
P_{mg} % range		10.6–51.3	727–1058	20.8–36.8
$\bar{x}$		27.8±3.3	886 ±30	29.3±1.3
GFR	inn	49.9±2.5	55.2±3.1	45.4±3.5
ml/min/100 g	den	50.0±2.3	57.2±2.3	50.4±3.8
Tm	inn	10.2±1.6	349 ±31	34.1±4.7
mg/100 ml GFR	den	6.5±1.3[a]	326 ±27[a]	26.6±3.8[a]

m = number of dogs, *n* = number of clearance periods, P = plasma concentrations of inorganic phosphate (Pi), D-glucose (G) and para-aminohippuric acid (PAH), respectively; GFR = glomerular filtration rate, Tm = maximal tubular transport of Pi, of G and of PAH, inn = innervated kidney, den = denervated kidney, [a] $p < 0.01$.

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Sensitivity of the developing chick myocardium to the positive inotropic effects of calcium and isoproterenol[1]

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Summary. The present results show that the sensitivity of the chick myocardium to the positive inotropic effect of Ca^{++} decreases during development and that the Ca^{++} concentration of the physiological solution used must be lowered below 'normal' to study the effects of positive inotropic agents in preparations from younger embryos. Isoproterenol elicits positive inotropic responses in 7–9-day embryonic ventricle and in newborn chick atria; however, the 4-day embryonic myocardium is unresponsive to isoproterenol.

Although the first studies on the effects of acetylcholine and epinephrine on the embryonic chick heart were reported more than 40 years ago, it was not until 1950 that BARRY[4] reported the first quantitative studies on isolated, spontaneously beating embryonic chick hearts. The present report deals with studies of the positive inotropic effects of Ca^{++} and isoproterenol on isolated, electrically paced preparations of embryonic and newborn chick myocardium.

Materials and methods. Hearts were isolated from embryos obtained from fertilized eggs (White Leghorn) at various times during development or from newborn chicks within 1 week of hatching. Most studies employed whole 7–9 day embryonic ventricle or newborn chick left

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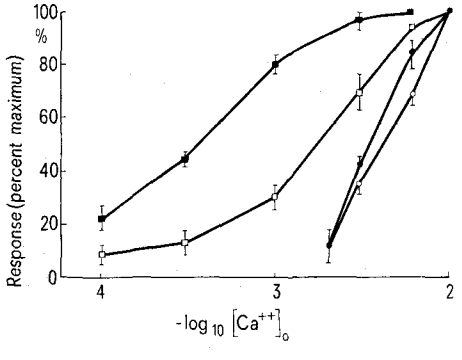
Sensitivity of the 7–9-day embryonic ventricle and newborn chick atria to isoproterenol in HEPES-bicarbonate buffered physiological solution

Preparation[a]	$CaCl_2$ (m*M*)	Baseline Force (% max)	l-Isoproterenol		
			EC_{50}[e]	Increase (%)	Max (% of Ca^{++} max)[c]
7–9-day Embryo	0.6	39.4±4.3 (7)[d]	$2.2\pm0.65\times10^{-8}$ (7)	111± 19 (7)	79.7±4.2 (7)
7–9-day Embryo	2.0	94.5±5.4 (4)	–[b]	3.5±3.5 (4)	–
Newborn chick	2.0	30.8±3.6 (4)	$1.0\pm0.27\times10^{-8}$ (5)	131.6± 32 (5)	61.0±5.3 (5)

[a] All preparations were paced at 180 beats/min. [b] Indeterminate due to small response. [c] Maximum force was determined by adding $CaCl_2$ in 1 m*M* increments at the top of the isoproterenol dose-response curve. [d] All values are expressed as the means ± SEM. The numbers in parentheses refer to the number of determinations. [e] Concentration of isoproterenol which produces 50% of the isoproterenol maximum response.

atrium. The various preparations were bathed in a bicarbonate buffered physiological solution (120 m*M* NaCl; 4.8 m*M* KCl; 0.6 m*M* KH_2PO_4; 0.6 m*M* $MgSO_4$; 5 m*M* glucose; 25 m*M* $NaHCO_3$; and variable amounts of calcium) or in a HEPES-bicarbonate buffered solution which was the same as the above except it contained a lower $NaHCO_3$ (11.9 m*M*) and 10 m*M* HEPES buffer, pH 7.4 at 37 °C. Both solutions were maintained at 37 °C and bubbled with 95% O_2;5% CO_2. The pH of the HEPES-bicarbonate physiological solution was more stable and slightly lower than that of the other bicarbonate buffered solution (pH 7.4 as compared to pH 7.6–7.7). Embryonic preparations were paced by field stimulation; newborn chick heart preparations (atria or ventricular strips) were paced with bipunctate electrodes (all at 180/min). Force of contraction was measured with LVDT (embryos) or force displacement transducers (newborn chick). All dose response curves were performed using the cumulative technique and responses are expressed as percent of maximum.

Results and discussion. Most physiological solutions contain 1.5–2.5 m*M* $CaCl_2$. Our initial attempts to perform cumulative dose response curves to isoproterenol on 4-day embryonic heart preparations bathed in a physiological solution containing 2.0 m*M* $CaCl_2$ were unsuccessful and prompted us to determine the sensitivities of our various preparations to Ca^{++}. These experiments were performed in the bicarbonate buffered solution and are summarized in the Figure. The sensitivity of the chick myocardium to the positive inotropic effect of Ca^{++} decreases quite markedly during development. BARRY et al.[5] have reported somewhat similar results in that the percent increase in force development by strips of chick ventricular myocardium when the Ca^{++} concentration is increased from 1.8 to 3.6 m*M* increases as the age of the embryo increases.



Dose-response curves to Ca^{++} in embryonic and newborn chick myocardium. 4-day embryo (■), 7–9-day embryo (□), chick atrium (●), chick ventricle (○). The mean ± SEM is shown for each concentration studied.

The Table summarizes the pertinent data from experiments in which dose response curves to l-isoproterenol were performed on 7–9-day embryonic ventricle and chick left atria preparations bathed in the HEPES-bicarbonate medium. Three points should be noted: 1. dose-response curves to isoproterenol in 7–9-day embryonic chick ventricle could only be performed in the presence of a reduced Ca^{++} concentration; 2. the sensitivity to the positive inotropic effect of isoproterenol is similar in the two types of preparations; and 3. the sensitivity to Ca^{++} appears to be greater in the HEPES-bicarbonate physiological solution. We were not able to demonstrate a positive inotropic response to isoproterenol in 4-day embryonic hearts under any conditions (various solution at different Ca^{++} concentrations and pHs).

In a preliminary communication from our laboratory[6] we reported that the 7–9-day embryonic ventricle is considerably less sensitive to the positive inotropic effect of isoproterenol than is the newborn chick myocardium. This conclusion was based on a very large number of experiments performed in the bicarbonate buffered solution under conditions exactly as we have used in the experiments reported herein. During further studies, the sensitivity of the 7–9-day embryo preparations increased abruptly to that reported in this communication. The sensitivity to isoproterenol in the newborn chick myocardial preparations and to Ca^{++} in embryonic and newborn chick preparations has remained constant during all of our experiments. Some workers have presented strong evidence that isoproterenol elicits positive inotropic responses in 4-day embryonic chick hearts[7] while others have presented equally good evidence that these preparations are not responsive to catecholamines[8].

We have performed a considerable number of experiments to determine if a seemingly trivial alteration in procedure markedly affects the sensitivity of 7–9-day embryonic heart preparations to isoproterenol. The sensitivity (EC_{50}) to the inotropic effect of isoproterenol was not affected by: 1. the rate the preparation is driven (120–240

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beats/min); 2. the Ca^{++} concentration (in the narrow range that can be studied); 3. the pH of the bathing medium (7.0–7.8, buffered with bicarbonate, bicarbonate-HEPES, or phosphate); and 4. temperature (30° or 37 °C). Furthermore, the sensitivity of hearts from embryos obtained from eggs laid by hens at the beginning and end of their productive period did not differ. The contractility of preparations stabilized in a phosphate buffered medium aerated with 100% O_2 deteriorated if the medium was then bubbled with air, indicating that the preparations do not suffer from O_2 toxicity under our routine conditions of study. Therefore, it seems unlikely that methodological differences are the basis for the conflicting results that have been obtained in different laboratoires, or within our own laboratory. It would seem more probable that the different results that have been reported by our own and other laboratories are due to true differences in the preparations studied.

Influence of larval diapause on pheromone communication in the Khapra beetle, *Trogoderma granarium* Everts

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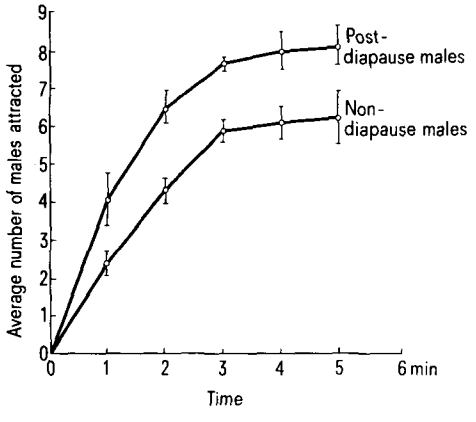
Summary. In *Trogoderma granarium*, induction of larval diapause by sub-optimal temperature enhanced the efficiency of pheromone perception by adult males. Such diapause also altered the pattern of pheromone production by females.

In pheromone communication among insects, release of the chemical stimulus, as well as the response of the target insect, is influenced by a variety of factors[1]. In our earlier studies, we had observed that in *T. granarium* the female secretes a pheromone which attracts the male, and that factors such as male age, prior mating and presence of females affect the male's response to the pheromone[2]. The larva of this beetle undergoes a facultative diapause under suboptimal temperatures[3]. Such diapausing larvae feed intermittently, resulting in the accumulation of fat, glycogen and protein[3,4]. Further, post-diapause females have been shown to have higher fecundity[5]. The present study relates to the influence of this type of diapause on the adult behaviour of *T. granarium* in terms of pheromone production by females and the responsiveness of males to the pheromone.

Materials and methods. The insects used in this study were obtained from a stock culture maintained for several generations at 36 ± 1 °C on broken wheat. For inducing diapause, 18–20-day-old larvae from the stock culture were released on fresh medium and maintained at 25 ± 1 °C. After a period of 4 months, the larvae were returned to 36 °C to break the diapause. On pupation, males and females were separated and kept for adult emergence.



Response of post-diapause males to female sex pheromone.

Since the behavioural response of an insect to sex pheromone bears a direct relationship to the pheromone content over a range of concentrations, the level of response of males was considered a reliable indicator of the quantity of pheromone secreted in unit time[6,7]. For collection of pheromone, 3 batches of freshly eclosed females from both post-diapause and normal lots were released separately in petri dishes lined with absorbent paper. The papers were removed at pre-determined intervals and fresh papers were provided till the insects started dying in any of the replicates. Pheromone from each such paper was extracted separately in known volume of diethyl ether. Each extract was bioassayed in an olfactometer using 6–7-day-old virgin males obtained from the stock culture. For comparing the level of responsiveness of post-diapause and normal males to the female pheromone, 6–7-day-old males from these 2 categories were assayed with a known concentration of stock pheromone extract. The number of males attracted to the pheromone source was scored every minute for 5 min. All assays were repeated 10 times with 10 fresh males per assay. The olfactometer and the method of assay employed in these studies were the same as described by Adeesan et al.[2].

Results and discussion. The pattern of pheromone secretion, by both post-diapause and normal females, in relation to their age was significantly different (table). In the case of normal females, pheromone secretion was highest during the first 7 days after eclosion and subsequently the secretory activity diminished. By about the 12th day, the females started dying. However, in post-diapause females maximal secretory activity was observed up to 14 days post emergence. Even after this period, the females continued to secrete the pheromone up to 18 days, when they started dying.

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