chickens maintained in tissue culture were harvested and inoculated into test tubes containing coverslips. When the cells had formed a moderately heavy sheet, the coverslips were removed from several tubes inoculated with a given tumor cell or transformed cell line, fixed and stained by the method of KLINGER and LUDWIG[10] for microscopic examination.

The percentage of cells containing sex chromatin was determined by examining the cells on several coverslips prepared from each culture, as noted in the Table. The transformed chick embryo cells appeared to be of male origin as they showed sex chromatin in 3.5% of the cells, thus falling within the range of 1.0 to 5.4% which KOSIN and ISHIZAKI[6] had shown to be characteristic of male cells from the base of a growing feather in 3-week-old New Hampshire chickens, whereas female cells showed sex chromatin in from 35 to 52%. In all male or female chickens, the cells cultivated from the tumors induced by the transformed male cells showed the male pattern of sex chromatin, indicating that the tumor was composed mainly of the male donor cells injected. On the other hand, the tumor cells from sarcomas induced by inoculation of virus showed the sex chromatin pattern characteristic of the sex of the host chicken.

These results indicate that chick embryo cells infected with Rous sarcoma virus *in vitro* are malignant and produce sarcomas when introduced into the wing web tissues of the same inbred strain of white Leghorn chickens from which the embryonic tissue was obtained.

Sex Chromatin in Cells

Source of cells			Number of cells counted	% of cells containing sex chromatin
Virus-transformed chick embryo donor cells			200	3.5
Transformed-cell-induced tumor				
	from male	1	200	2.0
		2	200	3.0
		3	200	7.0
		4	200	1.0
	from female	1	200	4.0
		2	200	4.0
		3	200	4.0
		4	200	3.0
Virus-induced tumor				
	from male		300	3.6
	from female		300	43.6

Résumé. Des cultures de cellules d'embryons de poulet mâles infectées avec le virus du sarcôme de Rous sont cancérigènes lorsqu'elles sont inoculées dans l'aile de poulets de la même souche Leghorn ('inbred'). Les sarcômes produits dans l'animal et les cellules transformées en culture ont la même chromatine sexuelle mâle, même si les tumeurs se sont développées dans des femelles. Au contraire, si les tumeurs sont produites par le virus, et non pas par des cellules infectées, la chromatine est du même sexe que l'animal récepteur.

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Effects of Calcium on the Spontaneous Contractions of the Isolated Ventricle of the Snail *Helix pomatia*

During the last decade it has been postulated that calcium ions play an essential part in the development of tension by a muscle by forming a link between electrical phenomena at the fibre membrane (excitation) and the mechanical response (contraction)[1–5]. A second action of calcium is to stabilize an excitable membrane by raising the threshold to stimulation[6–8]. Molluscan hearts do not in general respond to alterations of calcium concentration in the same way as do vertebrate hearts. For example, in the spontaneously contracting frog's heart calcium-rich solutions lead to systolic arrest[9], and calcium-deficient solutions to diastolic arrest[10], whilst in *Helix* the opposite occurs[11]. The effects of calcium on the spontaneous activity of *Helix* ventricular muscle have therefore been examined to see whether calcium has a fundamentally different action in this tissue compared with that in other (particularly vertebrate) heart muscle. The results obtained so far agree in the main with current ideas about the action of calcium, for calcium-rich solutions favour the development of tension and calcium-deficient solutions increase the excitability of the tissue.

Spontaneous contractions of the ventricle were recorded isometrically by a mechano-electric transducer (RCA 5734) after mechanical reduction by a spring-loaded lever. With this system the maximum shortening of the ventricle was never more than 5% of the resting length. Maximum tension was developed during a contraction when the ventricle was stretched to produce a resting tension of 1–2 g. The physiological saline contained (*mM*): Na^+ 85, K^+ 4, Ca^{2+} 9, Mg^{2+} 14, Cl^- 130, HCO_3^- 5.

As the contracting ventricle is sensitive to increases in the tonicity of the saline, the effects of calcium-rich solutions had to be studied in a saline with reduced sodium chloride concentration. These experiments were controlled by others in which the effects of low sodium chloride concentration alone were determined, isotonicity being maintained with sucrose. When the calcium chloride concentration was reduced, isotonicity was maintained by either sodium chloride or sucrose.

The ventricular contraction rate increases as the calcium concentration is decreased, and calcium chloride can usually be omitted from the saline without inhibiting the spontaneous contractions. ('Calcium-free' saline actually corresponds to severe depletion of calcium only as no

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special precautions were taken to remove the last traces of this ion from the other salts used in the saline, or from saline adhering to the preparation when the solutions were changed). The relationship between maximum tension developed during a contraction, the contraction rate, and low-calcium concentrations is shown in Figure 1. The response is similar whether isotonicity is maintained by sodium chloride (Figure 1a) or by sucrose (Figure 1b), indicating that neither the small increase in sodium concentration in the one nor the reduction in total chloride concentration in the other has any intrinsic effect. The increase in the contraction rate is due to a shortening of the diastolic period; the rates of rise and of decay of tension remain unchanged.

In calcium-free saline the diastolic tone increases (contracture), but contractions were inhibited in only one preparation. Possibly the calcium remaining in the bath is sufficient to preserve contractility. It has been shown that only a small amount of calcium is needed in the solution bathing a frog's muscle to enable it to develop tension[3]. A second factor may be the very high intracellular calcium concentration ($> 100\ mM$)[12].

Increasing the calcium concentration at the expense of sodium leads to an increase in the maximum tension developed during each contraction, and at very high calcium concentrations (36–45 mM) to a decrease in the contraction rate. The relationship between contraction rate, tension, and excess calcium concentration is shown in Figure 2. The effect of low sodium concentrations alone is to produce a slowing of the contraction rate, but no increase in tension; often the contractions become irregular, and at very low sodium concentrations they may be inhibited altogether.

Calcium can compensate for moderate decreases in sodium concentration. It was regularly observed that with the calcium concentration increased to 18 mM, and the sodium concentration reduced by 27 mM, no decrease in contraction rate was seen, although a similar reduction of sodium concentration alone did lead to a decreased contraction rate. At higher calcium concentrations a decrease in rate was observed, which could be due to either the damping effect of calcium on the excitability of the tissue, or to the calcium being unable to completely compensate for the simultaneous decrease in sodium concentration. It was not possible to positively distinguish between these two possibilities, but none of the irregularities of the contractions that frequently accompany a change to low sodium concentrations alone were seen if the calcium concentration was raised simultaneously.

The shape of the recorded contraction alters as the calcium concentration is raised. Contractions recorded from a preparation in normal saline and in saline with 45 mM calcium are shown in Figure 3. The two types of contraction differ in that (a) the rate of rise of tension is greater in the calcium-rich saline, being in this instance 1.25 g/sec compared to a rate of 0.97 g/sec in normal saline, and (b) the relaxation time is longer in calcium-rich saline (6.6 sec compared to 4.3 sec in normal saline).

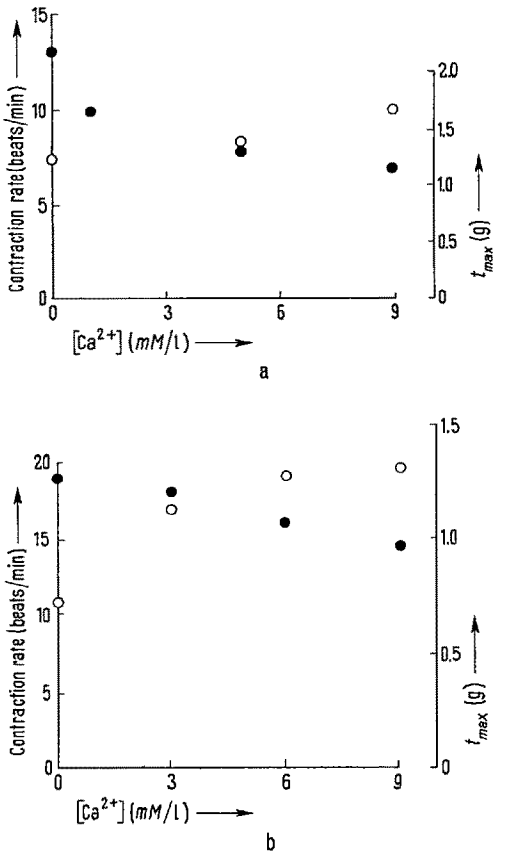

Fig. 1. The relationship between low-calcium concentrations, contraction rate and the maximum tension of each contraction (t_{max}). (a) Tonicity maintained with sodium chloride (3 mM NaCl for every 2 mM decrease in $CaCl_2$). (b) Tonicity maintained with sucrose. Closed circles show the contraction rate and the open circles the tension.

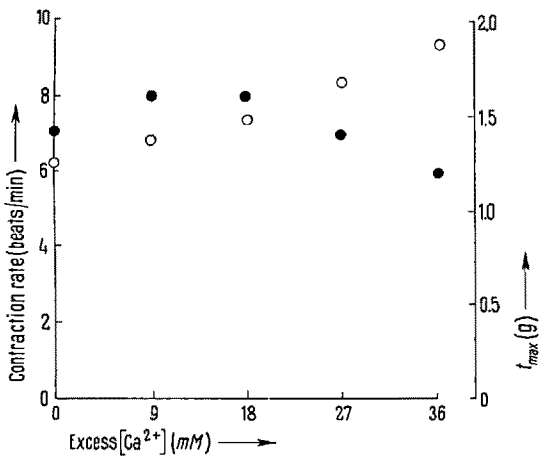

Fig. 2. The relationship between excess calcium concentration (normal is 9 mM), contraction rate and maximum tension of each contraction (t_{max}). Isotonicity preserved by reducing the sodium chloride concentration by 3 mM for every 2 mM increase in calcium chloride concentration. Closed circles show the contraction rate and open circles the tension.

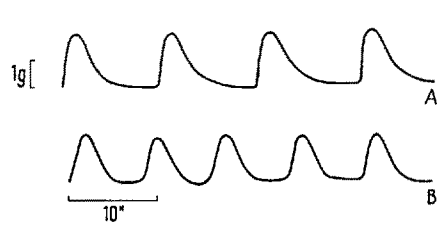

Fig. 3. The shapes of contractions recorded from a ventricle in A, saline with 45 mM Ca^{2+} and 31 mM Na^{+}, and B, normal saline. Tracing of original record; isometric recording.

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The difference in the response of frog and snail hearts to variations in calcium concentration does not seem to be due to a fundamental difference in the action of calcium on the respective myocardia. The effect of calcium on both contraction rate[10] and strength of contraction[13] in the frog is similar to that in *Helix*. The only striking difference is in the effects of calcium on the 'tone' of the heart.

In an electrically driven frog ventricle increases in calcium concentration are not followed by increases in resting tension, but the peak tension of the contraction is increased and the contraction is prolonged[13]. In the spontaneously contracting frog heart the increase in 'tone' is due to fusion of succeeding contractions. In *Helix* ventricle the contractions never fuse. The simultaneous decrease in contraction rate is sufficient to keep each contraction separate.

According to the hypothesis of JULLIEN et al.[14–16] the action of calcium in *Helix* ventricle is to stabilize the muscle fibre membrane by promoting the binding to the membrane of an unknown compound in an oxidised state that is an intermediary in the oxidative metabolism of the cell, and which has an inotropic effect when released into the perfusion fluid where it is reduced. With calcium-rich solutions the compound is firmly bound to the membrane and the inotropic action is absent, leading to 'diastolisation'. This action is antagonised by potassium, so that with low-calcium concentrations the action of potassium predominates, causing release of the compound and leading to 'systolisation'.

This hypothesis fails to account for the increase in tension with high-calcium concentrations which do not produce total failure, and for the fact that it is the resting tone that is increased by calcium-deficiency (contracture), whilst the individual conctractions are usually smaller. An alternative explanation is that calcium-rich solutions decrease the excitability of the membrane by hyperpolarizing it, causing the contraction rate to be reduced. The second (and independent) action of calcium is an inotropic one and is seen as long as spontaneous contractions persist. With calcium-deficient solutions the reverse would occur. A degree of depolarisation might be produced, making the membrane more excitable, and also producing contracture.

In the frog, low-calcium concentrations abolish contractility, electrical potentials still being recorded when contractions have ceased[10]. Thus if the myocardium is completely unable to contract no contracture is possible.

It seems that a higher concentration of calcium is needed to preserve contractility in the frog heart than in *Helix* ventricle. The high intracellular concentration of calcium in *Helix* ventricle might help to preserve contactility under conditions of severe calcium depletion.

Zusammenfassung. Untersuchungen über den Einfluss von Veränderungen der Calciumkonzentration auf die spontane *Helix*-Herzkammertätigkeit weisen darauf hin, dass Calcim hier keine Wirkung ausübt, die von seiner Wirkung auf Muskelgewebe im allgemeinnen grundverschieden ist. Hochkonzentrierte Calciumlösungen steigern die Kontraktionsspannung und bei höchsten Konzentrationen kann die Muskelerregbarkeit abnehmen, während schwachkonzentrierte Calciumlösungen die Muskelerregbarkeit erhöhen.

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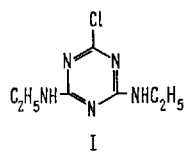
Beitrag zur Kenntnis der Resistenzphänomene einzelner Pflanzen gegenüber dem phytotoxischen Wirkstoff Simazin

Die phytotoxischen Verbindungen vom Typ des Simazins, 2-Chlor-4, 6-bis-äthylamino-s-triazin,
zeichnen sich durch eine ausgeprägte Wirkungslücke gegenüber Mais und verwandten Pflanzen aus und sind daher hervorragend zum selektiven Einsatz in dieser Kultur geeignet[1]. Es konnte gezeigt werden[2], dass Maispreßsaft befähigt ist, Simazin auf noch nicht genau abgeklärte Art abzubauen. Als Endprodukt des Abbaus in der intakten Maispflanze konnte in mit radiomarkiertem Simazin ausgeführten Versuchen Kohlendioxyd nachgewiesen werden, das den Tracer-Ringkohlenstoff enthielt[3,4].

Da der Maispreßsaft nach zweistündigem Erhitzen auf 80°C mit Simazin nicht mehr reagiert, schloss man zunächst auf eine enzymatische Natur dieses abbauenden Prinzips. Später konnte dann aber mittels eines Arbeitsganges, der die pflanzlichen Phenole erfassen sollte, aus Maispreßsaft eine Fraktion isoliert werden, die *in vitro* mit Simazin zu reagieren vermochte[5].

Nach längerem Stehen dieser Fraktion, die im Papierchromatogramm schon recht einheitlich erschien, schied sich ein Kristallisat ab, das durch Umkristallisation aus Methanol in schwach rosa gefärbte Prismen übergeführt werden konnte. Diese wiesen die folgenden Eigenschaften auf: (a) Schmelzpunkt: 159–162°C (Zers.). (b) Analyse[6]: gef. C: 50,99%; H: 4,47%; N: 6,59%; OCH_3: 14,69%. Für $C_9H_9NO_5$ ber. C: 51,18%; H: 4,30%; N: 6,63%; OCH_3: 14,70%. (c) Papierchromatographie: Whatman No. 4; absteigend; $FeCl_3$ als Entwicklungsreagens. Lösungsmittelgemisch: *n*-Butanol:Essigsäure:Wasser = 8:2:2, Rf-Wert: 0,83, Färbung: violett; Lösungsmittelgemisch: *n*-Propanol:NH_3(25%) = 7:3, Färbung: keine. (d) UV-Spektrum[6]: Lösung von 10 μg Substanz/ml Wasser: λ_{max} 262,5 mμ. (e) UV-Spektrum nach Kochen in Wasser[6]: Lösung von 10 μg Substanz/ml Wasser: λ_{max} 227,5 und 286,0 mμ.



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