Die untersuchten 6 Genine zeigen untereinander gegenüber einer Herzschädigung mit Barbiturat, Azid, Jodacetat, Mersalyl und Bariumchlorid (Tabelle I) kein unterschiedliches Verhalten. Die Zunahme der Herzleistung ist je nach Schädigungssubstanz verschieden, am stärksten nach Barbiturat, am schwächsten nach Jodacetat und Bariumchlorid.

Demgegenüber sind zwischen den einzelnen Geninen nach Schädigung mit Fluorid, N-Äthylmaleinimid, Jodacetamid und Phenylbutazon (Tabelle II) deutliche strukturabhängige Wirkungsunterschiede nachweisbar. Bei Fluorid und N-Äthylmaleinimid beziehen sie sich vorwiegend auf die 3,14-Dihydroxy-Verbindungen. Nach Fluorid ist Digitoxigenin stärker wirksam als Bufalin ($P < 0{,}01$), nach N-Äthylmaleinimid wirkt Bufalin stärker als Digitoxigenin ($P > 0{,}001$). Bei Fluoridgabe ist ausserdem 12β-Hydroxybufalin signifikant stärker wirksam als Bufalin ($P < 0{,}001$). Nach Jodacetamid, das in der untersuchten Konzentration einen positiv inotropen Eigeneffekt besitzt – erst höhere Konzentrationen vermindern die Herzleistung – wirken die drei Bufadienolide gleich stark; bei den Cardenoliden ist Digoxigenin dem Gitoxigenin ($P < 0{,}01$) und dem Digitoxigenin ($P = 0{,}05$) überlegen. Nach Schädigung mit Phenylbutazon haben bei den Di- und Trihydroxyverbindungen die Cumalin- und Butenolidrinderivate einen unterschiedlichen Effekt. Digitoxigenin ist dem Bufalin ($P < 0{,}02$), 12β-Hydroxybufalin und Desacetylbufotalin sind ihren entsprechenden Butenolid-Derivaten überlegen ($P < 0{,}01$ bzw. $P < 0{,}02$).

Da bei der vorliegenden Versuchsanordnung der Unterschied in der Wirkung der Genine auch auf einer unterschiedlichen Beeinflussung der Spontanfrequenz beruhen könnte, wurden die Veränderungen der Herzfrequenz und Kontraktionsstärke getrennt berechnet. Strukturabhängige unterschiedliche Effekte der Genine auf die Herzfrequenz waren nur bei Fluoracetat- ($P > 0{,}001$) und bei Fluoridgabe ($P < 0{,}001$) nachweisbar. Der in der früheren Mitteilung berichtete Unterschied nach Fluoracetatschädigung zwischen den 3,14,12-Trihydroxy-Derivaten und den beiden anderen Geninpaaren ist ausschliesslich auf eine unterschiedlich starke Herzfrequenzbeeinflussung zurückzuführen. Legt man der Auswertung nur die Kontraktionsamplituden zugrunde, wirken alle 6 Genine gleich stark. Nach Fluorid dagegen verstärkt die unterschiedliche Beeinflussung der Herzfrequenz die Wirkungsunterschiede zwischen den Geninen.

Nach den bisherigen Erfahrungen können als empfindlichste Schädigungssubstanzen zum Nachweis von Struktur-Wirkungsbeziehungen bei Geninen Chinin und Phenylbutazon empfohlen werden. Beide Verbindungen ermöglichen eine Differenzierung des Einflusses der Hydroxylgruppen und des Lactonringes. Untersuchungen mit Geninen, die verschiedene Substituenten an C_{10} und weitere Hydroxylgruppen besitzen, werden durchgeführt.

Die vorliegenden Befunde gestatten auch ein gewisses Werturteil über die optimal wirksame Konstitution. In der Mehrzahl der Versuche waren die Bufadienolide den Cardenoliden und die 3,14,12-Trihydroxy- den Dihydroxy- und 3,14,16-Trihydroxy-Verbindungen überlegen.

Summary. By means of guinea-pig auricle preparations, which were damaged in some way, it was demonstrated that bufadienolids compensate different model 'insufficiences' more efficaciously than cardenolids; and 3,14,12-trihydroxygenins more efficaciously than 3,14-dihydroxygenins, and even better than 3,14,16-trihydroxygenins.

W. FÖRSTER

Institut für Pharmakologie, Medizinische Akademie, Magdeburg (Deutschland), 19. April 1961.

Malignancy *in vivo* of Chick Embryo Cells Infected with Rous Sarcoma Virus *in vitro*[1]

Chick embryo cells infected with Rous sarcoma virus (RSV) *in vitro* form foci of altered cells[2,3] which continue to multiply and release virus and form a new, stable, transformed cell type[4,5]. It was of interest to determine if such cells possessed properties of malignancy *in vivo* independent of their capacity to release RSV which would produce tumors on release into the host tissues of the chicken. It was important therefore to determine the origin of cells in a Rous sarcoma induced by the injection of chick embryo cells which had been exposed to virus *in vitro* and developed the transformation characteristic of virus infection[2–5]. It has been shown by KOSIN and ISHIZAKI[6] that populations of chicken cells can be identified as of male or female origin by their content of sex chromatin, and recent studies by THIEDE[7] have demonstrated that by growing human embryonic cells in monolayers on glass coverslips *in vitro* the sex chromatin becomes prominent and permits a ready identification of the sex of the donor embryo by the technique of BARR and BERTRAM[8]. It was decided, therefore, to apply this technique to determine the origin of cells in Rous sarcomas which had been induced in chicks by the injection of cells transformed by virus *in vitro*.

Tumors were induced in the wing web of 2-month-old, white Leghorn cockerels and pullets by the injection into the wing web of 200 000 pock forming units of Rous sarcoma virus[9] or of 4.8×10^6 chick embryo cells which had been infected *in vitro* with the virus by the methods described by TEMIN and RUBIN[3]. Microscopic examination revealed that over 50% of these cells had undergone the characteristic morphologic transformation induced by the virus[3,5] and could be demonstrated to be releasing virus by the infective center assay technique of RUBIN[5]. The tumors produced by the injection of transformed cells appeared earlier and grew more rapidly than those induced by the virus. When the tumors reached a size of approximately 1 to 2 cm in diameter, they were removed from the wing web and the mucinous tumor mass was carefully dissected from the adjacent normal tissue. This tumor mass was digested with trypsin and cell suspensions prepared for cultivation by the methods described[3]. When a good monolayer of cells had grown out on the Petri dishes, the cells were removed with 0.05% trypsin and 600 000 cells inoculated into each of several test tubes containing coverslips and 2 ml of culture medium[3]. Aliquots of the transformed cells used for inoculation of the

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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.

H. R. MORGAN and A. P. ANDRESE

Department of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester (New York), March 28, 1961.

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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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