Uptake of aflatoxin B_1 by plastic materials (30 μM aflatoxin B_1 in phosphate buffer 10 mM, pH 7.3)

Material	Shape	Size (cm)	Initial amount of aflatoxin in solution (μg)	Aflatoxin adsorbed (%)					
				15′	30′	45′	1 h	2 h	6 h
Silicone	Tube	0.40 × 100	117.6	9	15	22	32	40	53
Silicone	Tube	0.20 × 100	29.4	11	19	24	37	44	70
Tygon	Tube	0.40 × 100	117.6	31	42	52	59	70	75
Tygon	Tube	0.24 × 100	42.3	33	56	59	68	75	85
Tygon	Tube	0.16 × 100	23.5	60	73	76	77	80	88
PVC	Tube	0.40 × 100	117.6	22	28	36	42	62	70
PVC	Tube	0.10 × 100	7.4	34	40	43	53	67	75
Polyethylene	Tube	0.40 × 100	117.6	7	10	13	16	22	33
Plexiglass	Tube	0.80 × 36	169.2	0	0	0	0	1	1
Cellulose ester[a]	Filter	∅ 2.8	93.6	20	28	31	34	38	44
Cellulose ester[a]	Filter	∅ 2.4	93.6	14	22	26	28	32	36
Cellotate[a]	Filter	∅ 2.8	93.6	20	33	39	44	52	54
Cellotate[a]	Filter	∅ 2.4	93.6	13	24	30	35	44	47
Nylon	Filter	∅ 2.4	93.6	3	5	5	6	6	7
Regenerated cellulose[b]	Membrane	2.5 × 2.5	93.6	0	0	1	1	1	1
Teflon	Sheet	6.25 × 1.0	93.6	0	1	2	2	2	3
Parafilm[c]	Sheet	6.25 × 1.0	93.6	5	6	6	6	6	6

[a] Millipore Filter Corporation, Bedford (Mass., USA). [b] Bel-Art Products, Pequannock (N.Y., USA). [c] American Can Co., Neenah (Wisc., USA).

and in hepatic microsomal preparations, interactions with DNA and serum proteins, inhibition of DNA-directed RNA synthesis and DNA synthesis.

Preliminary experiments showed that aflatoxin B_1 could be rapidly taken up by soft plastic materials used in several analytical procedures. Research was started to ascertain the materials most suitable for circulating and filtering aqueous solutions of aflatoxin B_1.

The results, summarized in the Table, show that uptake of aflatoxin by soft plastic materials is a rapid and extensive phenomenon. It can be a very important source of error in most of the experimental work carried out with these lipophilic mycotoxins.

Zusammenfassung. Aflatoxin wird von einigen gebräuchlichen Kunststoffen innerhalb kurzer Zeit in verhältnismässig grosser Menge absorbiert. Die vorliegenden Ergebnisse erlauben es, analytische Fehler bei Verwendung ungeeigneten Materials zur Zirkulation oder Filtration von Aflatoxinlösungen zu vermeiden.

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Biology Division, C.C.R. Euratom, Ispra (Italy), 17 November 1970.

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Relationship Between the Potentiation of Potassium-Induced Contracture of Cardiac Muscle by Four Cardenolides and their Inhibitory Effects on the Sodium Potassium Activated Adenosine Triphosphatase of Brain

In view of the current discussion of a close relationship between the positive inotropic action of cardiotonic steroids and their inhibitory action on the Na, K-activated ATP-ase[1], these two actions were measured and compared with digitoxigenin and 3 derivatives. A clear correlation was found as stated below.

In a previous paper, we reported that the cardiotonic activity of digitoxigenin was profoundly affected by introducing a hydroxy group or an oxo group in position 15. The order of the potency was: digitoxigenin (I) > 15β-hydroxydigitoxigenin (II) > 15-oxodigitoxigenin (III) > 15α-hydroxydigitoxigenin (IV), the last being practically inactive[2]. These 4 compounds were used in the present study. The compounds were kindly supplied by Dr. M. Okada of Tokyo Biochemical Research Institute, Tokyo.

Potentiation of potassium contracture of the cardiac muscle. The cardiotonic activity was measured by a new assay method which has been developed by Takeda et al.[3]. The method makes use of the potentiation of potassium contracture of the frog ventricular muscle by cardiotonic steroids.

Frogs, *Rana nigromaculata*, were used. A strip of the ventricular muscle was dissected and mounted in a bath which contained 3 ml of Ringer's solution (NaCl 111 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM, $NaHCO_3$ 1.2 mM, glucose 2.7 mM), which was aerated by oxygen. The muscle was stimulated electrically at a rate of 0.2 cps throughout

the experiment, except when potassium contracture was induced. By means of a strain-gauge transducer, a carrier amplifier and a DC-amplifier, the contraction of the muscle was recorded isometrically with an ink-writing oscillograph. Stock solutions were prepared by dissolving the compounds tested in 70% ethanol in a concentration of 1 mg/ml. By pilot experiments, low and high doses were determined for each compound, e.g. I (0.12 and 0.36 μg/ml), II (1.80 and 5.40 μg/ml) and III (10.0 and 30.0 μg/ml), the ratio of high dose to low dose being 3.

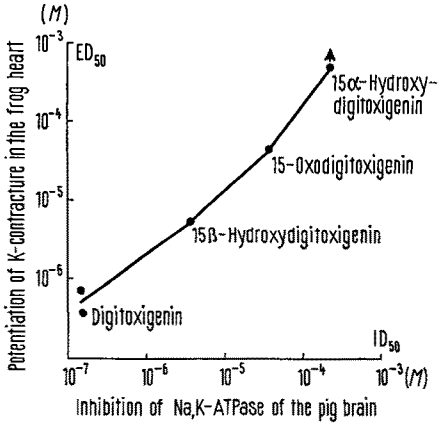
In short, the assay procedure was as follows: 1. Replacement of the bathing solution with Ringer's solution containing the compound tested in the low concentration brought about a gradual increase in twitch tension of the muscle, which reached a steady higher level in 40–45 min. 2. The electrical stimulation was interrupted, and potassium contracture was induced by replacing the bathing solution with a modified Ringer's solution, which contained 60 m*M* K. 3. The bathing solution was replaced by Ringer's solution containing the compound in the high concentration, and the same procedures as 1. and 2. were repeatet. The extent of the potassium induced contracture was expressed as percentage of the maximal contracture tension, which was induced by a modified Ringer's solution containing 110 m*M* K and 5 m*M* Ca. In each modified Ringer's solution, equivalent amount of NaCl was replaced by KCl.

Compounds I and II were assayed with the same group of frogs, 4 frogs being allocated to each compound. Compounds I and III were assayed with another group of frogs in the same way. In both sets of assay, compound I served as the reference standard, and ED_{50} (the concentration of the drug necessary to produce 50% of the maximal contracture tension of the muscle) and the relative potency of each compound were obtained according to the parallel line assay technique.

Inhibition of the Na, K-activated ATP-ase of the pig brain. The effects of these compounds on Na, K-activated ATP-ase activity were examined with a partially purified enzyme preparation. The enzyme preparation was obtained from the pig brain microsome fraction by the treatment with a high concentration of sodium iodide[4]. The enzyme preparation was practically inactive in the absence of sodium and potassium ions. Na, K-ATP-ase activity was measured according to the procedure of NAKAO et al.[4].

Relation between the two activities. As shown in the Table and the Figure, the 2 kinds of activities of the 4 cardenolides are well correlated each other. This, on the one hand, provides additional evidence for the discussion cited above on the mode of action of cardiotonic steroids. On the other hand, the results confirm quantitatively the significance of oxygen functions at position 15 with regard to their structure activity relationship.

Recently the same order of potency was obtained by REPKE[5] regarding the inhibitory activity of these compounds on Na, K-activated ATP-ase of the guinea-pig heart.



Relation between the effects of digitoxigenin and 3 derivatives on K-contracture of the frog ventricular muscle (ordinate) and those on Na, K-activated ATP-ase of the pig brain (abscissa). ED_{50}, ID_{50}, same as in the Table. Arrow indicates that the point should be some higher (see the Table).

Comparison of the activity in potentiating K-contracture of the frog ventricular muscle and that in inhibiting Na, K-activated ATP-ase of the pig brain among digitoxigenin (I) and its derivatives (II–IV).

	Potentiation of K-contracture		Inhibition of Na, K-activated ATP-ase
	ED_{50} (*M*)		ID_{50} (*M*)
I	3.80×10^{-7} [a]	7.15×10^{-7} [b]	1.5×10^{-7}
II	5.44×10^{-6} [a]		3.6×10^{-6}
III		4.62×10^{-5} [b]	3.6×10^{-5}
IV		$> 5 \times 10^{-4}$ [c]	2.2×10^{-4} [d]
	Relative potency		
I	1.00 [e]		1.00 [e]
II	0.070 (0.057∼0.085 [f])		0.042
III	0.016 (0.012∼0.020 [f])		0.0042
IV	(< 0.0016) [c]		0.0007

ED_{50}, concentration of a compound necessary to produce 50% of the maximal contracture tension of the muscle. ID_{50}, concentration of a compound producing 50% inhibition of the enzyme activity. [a] Compounds I and II were assayed with the same group of frogs. 4 frogs were allocated to each compound. [b] Compound I and III were assayed with another group of frogs. [c] The value was estimated from the previous experiment with isolated frog hearts (Straub's preparation)[2]. [d] The value was obtained by extrapolation of the concentration-activity curve. [e] Compound I served as reference standard. [f] 95% confidence limits.

Zusammenfassung. Bei Digitoxigenin und 3 weiteren Derivaten wurde die Stärke der potenzierenden Wirkung auf die Kalium-Kontraktur des Froschherzmuskels und der hemmende Effekt auf den Transport der ATPase des Schweinehirns verglichen und eine eindeutige Korrelation zwischen diesen beiden Wirkungen festgestellt.

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