Every point in the Figure 1 represents an average of at least four experiments. Because in the previous experiments[2,3,7] the linear relationship between the logarithms of concentrations and % control activities in the explored region and the parallelism of these curves was demonstrated, it was possible to express the relative potencies of cardiac glycoside as a distance between the curves on the ordinate 50, that is as a potency index 50. We compared the pI 50 of the different cardiac glycosides with their mean lethal doses in the cat expressed in mg/kg[8]. It may be noted from the Figure 2 that there is a direct relationship between the mean lethal doses and pI 50 of the cardiac glycosides, the correlation coefficient being 0.99 ($p = 0.001$). In contrast to the findings of SOLOMON *et al.*[1], this direct relationship is valid also for the lanatoside-B, when investigated on cold-stored erythrocytes.

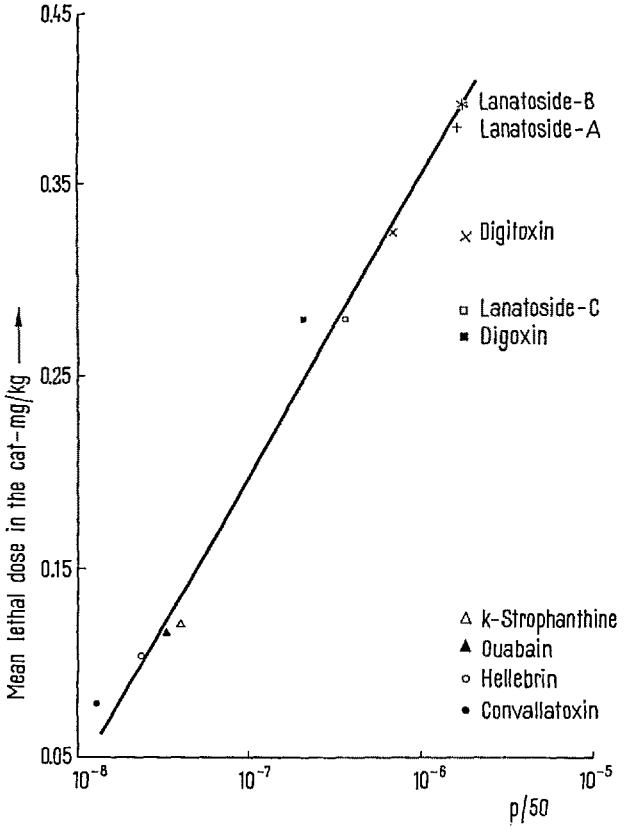

Fig. 2

On the basis of our results and those of the other authors[1-3,6], we suppose that the determination of the doses of cardiac glycosides necessary to produce a 50% blockade of the active potassium reentry into the incubated red cells may be used for evaluating potencies of the glycosides investigated. If the hypothesis about the mechanism of the cardiotonic action of cardiac glycosides by influencing the electrolyte metabolism should prove correct, the proposed method would actually be specific. At the same time, our results support this hypothesis. The quantity of the glycoside that is necessary to determine its potency is minimal and the method is relatively simple and inexpensive. In contrast to the methods currently used of evaluating the effectiveness of cardiac glycosides on animals, which are characterised by a great variability of results, we did not see a similar variability in the case of the method described.

J. MACHOVÁ[9]

Pharmacological Laboratory, Chemical Institute, Slovak Academy of Sciences, Bratislava (Czechoslovakia), March 29, 1960.

Zusammenfassung

Bei neun untersuchten Herzglykosiden konnte eine direkte Beziehung zwischen der Kardiotoxizität und dem Grad der Beeinflussung des aktiven Kaliumtransportes festgestellt werden.

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[9] The able assistance of Mrs. G. TÓTHOVÁ is gratefully acknowledged. Statistical analysis of the data was performed by Ing. R. ŠTUKOVSKÝ. Digitoxin, ouabain, and *k*-strophanthine were kindly supplied by E. Merck A.G., digoxin by C. F. Boehringer & Söhne GmbH and Lanatoside-A,- B, -C by Richter Gedeon.

Further Observations on the Effects of Selenium and Antioxidants on Exudative Diathesis in Chicks

The ability of trace amounts of dietary selenium to replace vitamin E under some experimental conditions has raised questions about the metabolism of the biologically active form of this element[1]. It is well established that some selenium compounds will completely replace vitamin E in preventing exudative diathesis in the chick[2,3], but there has, to our knowledge, so far been no information on the tissue storage of the biologically active selenium compound(s). Therefore, we have examined how long the protection by selenium against exudative diathesis would last after selenium was removed from the diet. Also, in continuation of previous studies[4] on the replacement of vitamin E by antioxidants, two substances not previously tested in this laboratory have been examined.

Two vitamin E-free basal diets, differing primarily in the protein source, were used. Diet 2304 contained 60% Torula yeast 3N (Lake States Yeast Corporation, Rhinelander, Wisconsin), 3% gelatine, 5.17% salt mixture no. 4[5], 0.1% B vitamin mixture[5], 0.2% choline chloride, and 31.53% sucrose. Diet 2343 contained 30% of isolated soybean protein (ADM Assay Protein C-1, Archer-Daniels-Midland), 5.17% salt mixture no. 4[5], 0.1% B vitamin mixture[5], 0.2% choline chloride, 1% lard, and 63.53% sucrose. The diets were supplemented with 1 mg% dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (Synkavit «Roche»). Vitamins A and D_3 in aqueous solution[6] were given orally twice weekly. Dayold chicks (New Hampshire × White Leghorn) were fed a commercial starter ration for the first week and then placed in groups of 9 or 10 and given the experimental diets. They were examined daily for exudates.

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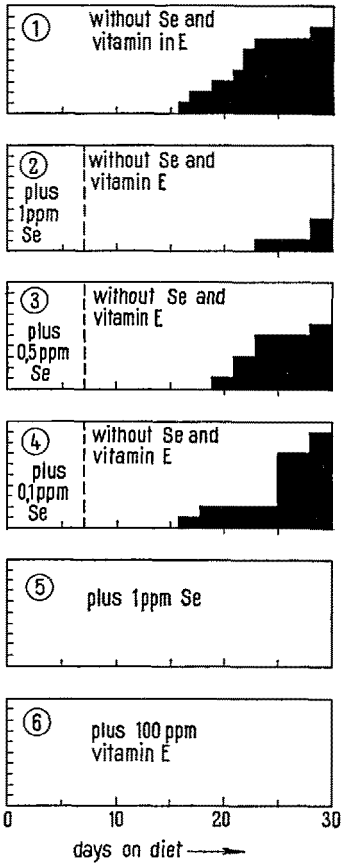
[6] H. DAM, S. HARTMANN, J. E. JACOBSEN, and E. SØNDERGAARD, Acta pysiol. scand. *41*, 149 (1957).

Selenium study. The purpose of this study was to determine the capacity of the chick to store dietary selenium, using the time for appearance of exudates as the criterion of the rate of depletion. One group received the Torula yeast diet 2304 alone while control groups received either 1 ppm selenium or 100 ppm DL-α-tocopheryl acetate (Ephynal «Roche») in this diet for the entire experimental period. Three additional groups received 1, 0.5, or 0.1 ppm Se, as SeO_2, in this diet for the first 7 days, at which time the selenium was withdrawn. The number of days required for exudates to appear in the various groups is shown in the accompanying figure. In the unsupplemented Group 1, the first exudate developed on the 16th day; by the 28th day, 8 of the 10 chicks had the deficiency symptom. In Groups 2, 3, and 4, fed the diets supplemented with 1, 0.5, or 0.1 ppm Se for the first week, the first exudates appeared after 23, 19, and 16 days of the experiment, respectively. After 28 days, 3, 6, and 9 of the chicks in these groups had exudates, and after 35 days (not shown in the figure), 9, 8, and 9 chicks had exudates. None of the control chicks receiving the diets supplemented with 1 ppm Se or 100 ppm DL-α-tocopheryl acetate (Groups 5 and 6) had exudates.



The effect of feeding selenium in a vitamin E-free Torula yeast diet for seven days on the subsequent rate of development of exudative diathesis. Ordinate scale indicates number of chicks with exudates (10 chicks/group).

It is obvious that the supplementation of the diet with 1, 0.5, or 0.1 ppm Se for the first 7 days had a delaying effect on the onset of exudative diathesis correlated with the selenium level fed. The delaying effect is especially marked for the group receiving 1 ppm Se. This indicates a certain storage of selenium in a biologically active form.

The level of 1 ppm Se is about twenty times the minimum amount required for consistent prevention of exudative diathesis (0.05 ppm)[7]. If a chick consumed an average of 10 g of feed/day during the first week, the first week's intake of selenium would be 3.5 γ at the level of 0.05 ppm and 70 γ at the level of 1 ppm. From the observation that one week of feeding 1 ppm of selenium delayed symptoms by only about 7 days, it is apparent that only a small fraction of the administered selenium was utilized for this purpose. Since these small amounts are very probably completely absorbed, it may be deduced that the tissues have a limited capacity for synthesizing and storing the biologically active form of selenium.

Antioxidant study. The results of two experiments in which the antioxidant Ethoxyquin[8] (1,2-dihydro-6-ethoxy-2,4-trimethylquinoline) was tested for its ability to prevent exudates are shown in the accompanying Table. In the first experiment, the basal soybean protein diet containing only 1% of lard produced a high incidence of exudates. Ethoxyquin completely prevented the exudates as did also DL-α-tocopheryl acetate and selenium dioxide. In the second experiment (Groups 11–14), the dietary supplements were not added until the groups had been on the basal diet for 7 days. This was done further to deplete the chicks of vitamin E and thus reduce the possibility that the action of the dietary antioxidant was through its sparing effect on residual tissue tocopherol. As can be seen, the Ethoxyquin was again very effective; only one chick in the group had exudative diathesis. Another antioxidant, 5-*n*-pentadecyl-resorcinol, which was found to be as active as α-tocopherol in reactivating aged preparations of cytochrome reductases[9], had no effect on the syndrome at the level of 0.05% of the diet (limited supply prevented further testing). The positive results obtained with Ethoxyquin are in agreement with reports by MACHLIN *et al.*[10].

Influence of Ethoxyquin on the Incidence of Exudative Diathesis

Experiment No.	Group No.	Diet	No. of exudates[a]	Average weight 21 days (g)
1	7	2343 (containing soybean protein)	7^{10}	225
	8	2343 + 0.01% DL-α-tocopheryl acetate	0^{10}	249
	9	2343 + 0.33 ppm Se (as SeO_2)	0^{10}	254
	10	2343 + 0.1% Ethoxyquin	0^{10}	190
2	11	2304 (containing Torula yeast)	9^{10}	163
	12	2304 + 0.01% DL-α-tocopheryl acetate	0^{9}	199
	13	2304 + 0.33 ppm Se (as SeO_2)	0^{9}	179
	14	2304 + 0.1% Ethoxyquin[b]	1^{10}	171

[a] After 32 days of experimental feeding. The superscripts represent the number of chicks in the group.
[b] Ethoxyquin was omitted the first 7 days.

E. SØNDERGAARD, J. G. BIERI[11], and H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen (Denmark), July 27, 1960.

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[9] C. J. POLLARD and J. G. BIERI, J. biol. Chem. *235*, 1178 (1960).
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[11] Fulbright Research Scholar 1959–1960. Permament address: National Institutes of Health, Bethesda (Maryland).

Zusammenfassung

Bei Fütterung von Küken mit einer Vitamin-E-freien Nahrung unter Zugabe von 0,1–1 ppm Selen in der ersten Woche wurde das Auftreten exsudativer Diathese 2–7 Tage hinausgeschoben. Es wird deshalb angenommen, dass die biologisch aktive Verbindung von Selen nur geringfügig deponiert wird.

Die von amerikanischen Forschern gefundene Schutzwirkung von Ethoxyquin gegen exsudative Diathese wird bestätigt.

Effects of Selective Intracranial Section and Stimulation of Vago-Accessory Roots I. Afferent Fibers from Lungs and Aortic Area

The effects of intracranial section of vagal and/or accessory (bulbar and spinal) roots as well as of electrical stimulation of their central stumps have been studied in 23 normal or midpontine dogs with the following results: (i) Bilateral section of the vagal root causes the appearance of the typical respiratory patterns which normally follow bilateral division of the vagal common trunk, and completely abolishes the reflex responses to over-inflation and over-deflation of the lungs. However, bilateral section of bulbar and/or spinal accessory nerve roots does not influence the previous patterns of breathing either preceding or following bilateral section of vagal root, nor are the reflex responses to volume changes of the lungs affected. (ii) Both positive and negative respiratory effects induced by low (10–30/sec) and high (100–300/sec) frequency stimulation of the central stump of the vagal common trunk[1] are no longer present after intracranial section of ipsilateral vagal root, and are duplicated by stimulation (with the same parameters) of the latter, while stimulation of bulbar and/or spinal accessory nerve roots is without apparent effects on respiration, over a large range of frequencies and voltages. (iii) The hypotensive response evoked by electrical stimulation of the central stump of the vagal common trunk (more marked on the left side), due to the activation of depressor fibers coming from the aortic arch or subclavian artery, is completely abolished by intracranial section of the ipsilateral vagal root. Moreover, stimulation of the central stump of the bulbar accessory root fails to induce any circulatory effects.

The above results seem to indicate that the accessory nerve roots do not contain afferent fibers from the lungs or the aortic area. These enter the medulla only through the vagal root. The clusters of ganglion cells scattered along the accessory nerve roots[2] are probably concerned with the proprioceptive control of the striate muscles innervated by the external (spinal) and internal (bulbar) *rami* of the accessory nerve.

L. Sperti and E. Xamin

Istituto di Fisiologia dell'Università di Padova (Italy), August 1, 1960.

Riassunto

Le radici bulbare e spinale del nervo accessorio non contengono fibre afferenti provenienti dal territorio polmonare e dall'area aortica. Queste entrano nel bulbo esclusivamente con la radice del nervo vago. Le cellule gangliari contenute nelle radici dell'accessorio sono probabilmente in relazione con il controllo propriocettivo dei muscoli striati innervati dai rami esterno (spinale) ed interno (bulbare) di questo nervo.

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Effects of Selective Intracranial Section and Stimulation of Vago-Accessory Roots II. Efferent Cardio-inhibitory Fibers

The effects of intracranial section of vagal and/or bulbar accessory roots, as well as of electrical stimulation of the peripheral stumps of these roots, have been studied in 21 normal or decerebrate dogs with the following results: (i) Electrical stimulation of either vagal or bulbar accessory root induces, at almost the same threshold value, a definite bradycardia; increasing current intensities lead-on stimulation of either nerve, although more readily of the former-to cardiac arrest. No effects on cardiac rhythm have been observed on stimulation of the spinal accessory root. (ii) The electrical stimulation of a rather large medullary area including the dorsal motor nucleus of the vagus nerve causes bradycardia or cardiac arrest, according to the current intensity and to the electrode position. After section of the ipsilateral vagal root, it is still possible to obtain, from the same points, cardio-inhibitory responses, though they are less marked. This residual effect is completely abolished by the section of the ipsilateral bulbar accessory root. (iii) In the normal animal, bilateral section of the vagal root completely releases the cardiac rhythm from the tonic inhibitory restraint, while bilateral division of the bulbar accessory root does not significantly affect it, either when it is performed before or after vagal root section. On the other hand, in those decerebrate preparations which displayed a marked degree of bradycardia, bilateral division of the bulbar accessory root was always followed by a conspicuous acceleration of the cardiac rhythm in both cases.

The above results seem to indicate that both vagal and bulbar accessory roots carry efferent cardio-inhibitory fibers, the former to a rather larger extent; those carried by the vagal root seem to be the ones primarily concerned in the maintenance of the so-called vagal tone in the normal animal while the accessory component seems to be, at rest, largely dispensable. During the severing of the vagal root, we cannot rule out the possibility of accidental lesions of the IX nerve root, as well, and of its proprioceptive fibers; this might explain the absence of cardio-inhibitory tone in the accessory bulbar root in the normal animal. But, whether the activity of the bulbar accessory root, observed in the decerebrate animal, is reflex (from the IX nerve) or automatic, it seems, in either case, to be the result of the increased activity of the cardio-inhibitory centre, probably due to a release phenomenon.

L. Sperti and E. Xamin

Istituto di Fisiologia dell'Università di Padova (Italy), August 1, 1960.

Riassunto

Fibre cardio-inibitrici sono contenute non solo nella radice del X° nervo, ma anche nella radice bulbare dell'accessorio, seppure in grado minore. Mentre nel preparato normale le fibre cardio-inibitrici dell'accessorio non sembrano prendere parte al mantenimento del cosidetto «tono vagale», nell'animale decerebrato sono responsabili, almeno in parte, del notevole grado di bradicardia che caraterizza questo preparato.