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ACCUMULATION AND RELEASE OF ^{3}H-DIGOXIN BY GUINEA-PIG HEART MUSCLE

BY

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Owing to the availability of radioactively labelled cardiac glycosides the determination of minute amounts of these drugs in biological material can now be carried out. Thus, investigations on the distribution and metabolism of cardioactive glycosides in the living organism have been greatly facilitated. Formerly these compounds could only be administered in toxic amounts in order to obtain tissue concentrations which were sufficiently high for chemical or physicochemical analysis. The sensitivity of radioactive analysis allows the investigation of the fate of therapeutic glycoside doses in laboratory animals as well as in humans (Okita, Talso, Curry, Smith & Geiling, 1955; Gonzalez & Layne, 1960; Doherty, Perkins & Mitchell, 1961; Doherty & Perkins, 1962; Marcus, Kapadia & Kapadia, 1964; Marks, Dutta, Gauthier & Elliott, 1964; Lage & Spratt, 1965; Katzung & Meyers, 1965; Abel, Luchi, Peskin, Conn & Miller, 1965; Marcus *et al.*, 1964; Marcus, Peterson, Salel, Scully & Kapadia, 1966). Although the tissue accumulation of radioactive glycosides by a few organs has been studied in whole animals and in humans (Bretschneider *et al.*, 1962; Dutta, Marks & Smith, 1963; Marks *et al.*, 1964; Marcus *et al.*, 1964, 1966), the uptake of the labelled drugs by isolated organs has not been investigated so far.

The present paper deals with a kinetic study of the accumulation and release of ^{3}H-digoxin by guinea-pig isolated atria. The uptake and loss of the drug by heart muscle was measured as a function of its concentration in the bath fluid. The influence of the organs' frequency of beating and that of different calcium contents of the medium was also established. The release of ^{3}H-digoxin was studied for different initial digoxin levels of the atrial tissue. Investigations on the exchange of tissue bound digoxin with that in the medium will be dealt with in a forthcoming paper.

METHODS

Guinea-pig isolated atria were obtained according to the procedure described by Hoditz & Lüllmann (1964). The organ bath consisted of a 500 ml. beaker, which contained 250 ml. of the incubation medium. In all experiments carbogen (95% O_2+5% CO_2) was vigorously bubbled through the solution. Oxygenation carried out in this way provided sufficient agitation so that additional stirring was unnecessary. All experiments were carried out at 30°. In one series of experiments, carried out in order to determine the influence of the frequency of beating on the uptake of ^{3}H-digoxin, isolated left auricles were used instead of atria. The auricles were stimulated with various frequencies (see Results). The atria were equilibrated during 30 min in an oxygenated Tyrode solution, which contained 1.2 m-equiv Ca^{2+}/l. Unless stated otherwise this Tyrode solution

has been used throughout the experiments. After equilibration the organs were incubated for various periods up to 4 hr in a Tyrode solution which contained different concentrations of ^{3}H-digoxin. The desired specific activities were obtained upon dilution of the radioactive stock solution (126 μc ^{3}H-digoxin, dissolved in 1 ml. abs. ethanol) with the appropriate amounts of inactive digoxin. During equilibration, incubation and also during the wash-out experiments the organs were stimulated electrically by means of a Grass S 4 H stimulation device. The frequency of contraction was 180/min. Guinea-pigs of either sex (200–400 g) were used. The atria prepared from animals weighing 300 g and 400 g averagely had a mean wet weight of 90 and 110 mg, respectively. In order to relate the wet weight of the atria and the animals' weight, the regression line of this relationship was established for 1,005 animals. The regression coefficient of this line proved 0.22 mg wet weight/g animal weight. Thus, the atrial wet weight increases by 0.22 mg for 1 g of animal weight (correlation coefficient = 0.9908). After incubation, the atria were pressed slightly between blotting paper for 90 sec, put into glass ampoules and weighed. To each weighed organ 3 ml. Hyamine* was added. The ampoules were sealed at the top and heated for 4–6 hr at 70°. The atria thus treated dissolved completely, yielding a homogeneous, transparent and slightly yellow solution. Digestion of the tissue was enhanced by occasional shaking. An aliquot sample (2 ml.) of the solution, which contained two-thirds of radioactivity present in the tissue was transferred to a glass counting vial, containing 10 ml. of a scintillation phosphor (4 g PPO + 0.5 g POPOP, dissolved in 1 l. toluene). After shaking, the samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer. By means of an internal tritium standard the individual quenching of each sample was corrected. The measured radioactivity of the organs was expressed as mμC/g tissue. The radioactivity of the medium was determined in each experiment.

In order to study the release of ^{3}H-digoxin, isolated atria were incubated for 2 hr in an oxygenated Tyrode solution, containing a given concentration of the labelled glycoside. Subsequently the atria were left for various periods (up to 3 hr) in a Tyrode solution which did not contain digoxin. Electrical stimulation was applied as usual. After incubation in the inactive Tyrode solution, the residual radioactivity of the organs was determined as described above. In order to investigate the effect of the various digoxin concentrations on the contraction amplitude of the atria, the contractions of electrically stimulated isolated atria were recorded in separate experiments via a transducer and a Helcoscriptor recording device, Type HE-86 t.

Chromatography

It had to be demonstrated that the counted tritium was related to ^{3}H-digoxin and not to a possible radioactive metabolite of the drug. Thus, atria which had been incubated in a ^{3}H-digoxin (5×10^{-7} g/ml.) containing solution for 2 hr as described above were homogenized in a 50% aqueous ethanol (approximately 4 ml./atrium). The homogenate was extracted several times with chloroform. The chloroform extract and the filtered aqueous ethanolic solution were both evaporated to dryness at reduced pressure. The residues thus obtained were respectively dissolved in 2 ml. anhydrous chloroform or in 2 ml. 96% ethanol. Both solutions (0.1 ml.) were subjected to thin layer chromatography (silica gel + $CaSO_4$, 0.2 mm layer). A mixture of ethyl acetate and n-butanol (9:1, v/v) was used for the development of the chromatogram. After development and drying the carrier material was divided into single fractions of approximately 1 cm^2 each, in the same direction as the solvent front had moved. The fractions were scraped off the glass plate separately. The silica gel of each fraction was transferred to counting vials, suspended in 2 ml. abs. ethanol and left for a few hours at room temperature. After addition of a liquid scintillation phosphor (4 g PPO $\pm$ 0.5 g POPOP, dissolved in 1 l. toluene) and shaking, the radioactivity of the samples was counted as usual. All radioactivity was recovered in one single fraction of approximately 1 cm^2, which had an Rf value of about 0.4.

Chromatography of inactive dixogin under identical circumstances yielded one single spot with the same Rf value. The digoxin spot was made visible upon exposure of the chromatogram to iodine vapour. Accordingly, the radioactive material extracted from the incubated atria most likely consists of ^{3}H-digoxin. The formation of radioactive metabolites under the experimental circumstances used in our experiments could not be demonstrated. Therefore, chromatographic data mentioned above suggest that the tritium measured may be related directly to ^{3}H-digoxin.

* Hyamine = p-(diisobutylcresoxyethyl)-dimethylbenzylammonium hydroxide, 1M in methanol.

RESULTS

Accumulation of ^{3}H-digoxin at various concentrations in the medium

The tissue uptake of ^{3}H-digoxin by isolated atria or isolated left auricles was studied for six different concentrations in the medium—that is, 5×10^{-8}, 1×10^{-7}, 2.5×10^{-7}, 5×10^{-7}, 1×10^{-6} and 2.5×10^{-6} g digoxin/ml. The lowest concentration did not influence the contraction amplitude of the isolated organs. In most cases the concentrations in the range $1–5 \times 10^{-7}$ g/ml. gave rise to an increase of contraction amplitude which was dose-dependent. In the majority of experiments these digoxin levels did not provoke contracture, even after prolonged periods of exposure. The two highest concentrations (1×10^{-6} and 2.5×10^{-6} g/ml.) showed a marked positive inotropic effect, which was followed by the development of contracture.

TABLE 1

EFFECTS OF VARIOUS CONCENTRATIONS OF DIGOXIN ON THE CONTRACTION AMPLITUDE OF GUINEA-PIG ISOLATED ATRIA

Digoxin concentration (g/ml.)	Increase of contraction amplitude in % of the initial value (mean of 5 expt.)	Onset of possible inotropic effect (min.) (mean of 5 expt.)	Contracture
5×10^{-8}	—	—	—
1×10^{-7}	14	5	—
$2{\cdot}5 \times 10^{-7}$	32	5	—
5×10^{-7}	36	3	In 1 out of 5 expt.
1×10^{-6}	87	1·5	In all expt.
$2{\cdot}5 \times 10^{-6}$	177	1	In all expt.

The effects of digoxin in the concentrations used in our investigations have been summarized in Table 1. The measured tissue radioactivity (mμC/g) tissue) after incubation in the tritium labelled digoxin solution was plotted against time, for each of the digoxin concentrations studied. Except for the highest concentrations investigated, the uptake occurred rather slowly, approximately 2 hr being required before the tissue radioactivity had reached equilibrium. The tissue/medium radio-activity ratios at equilibrium reached maximal values which were in the range 1.5–3. These ratios decreased when the medium concentration of digoxin was raised (Table 2). In order to allow a direct comparison of the results obtained for various digoxin concentrations, the

TABLE 2

UPTAKE OF ^{3}H-DIGOXIN AT VARIOUS CONCENTRATIONS. TISSUE/MEDIUM RADIOACTIVITY RATIO, TISSUE CLEARANCE, TIME REQUIRED UNTIL EQUILIBRIUM IS REACHED, $t_1/_2$ OF THE CELLULAR UPTAKE PROCESS, RATE CONSTANT AND RATE OF UPTAKE FOR EACH DIFFERENT DIGOXIN CONCENTRATION

Digoxin concentration in medium (g/ml.)	Tissue/medium radioactivity ratio (no correction for extracellular radioactivity	Tissue clearance (ml./g)	Approx. time required until uptake reaches equilibrium (min)	$t_1/_2$ of uptake process (min)	Rate constant k $k \times 10^{-1}$ (sec^{-1})	Rate of uptake ($g \times 10^{-7}$ digoxin/g cellular material/min)
5×10^{-8}	3·1	4·2	190	45	2·8	0·203
1×10^{-7}	2·9	3·9	180	36	2·8	0·465
$2{\cdot}5 \times 10^{-7}$	2·8	3·9	180	32	3·2	1·312
5×10^{-7}	2·0	2·6	170	22	6·4	3·045
1×10^{-6}	1·7	2·0	115	22	4·6	4·15
$2{\cdot}5 \times 10^{-6}$	1·5	1·7	55	7	16·0	41·68

cellular uptake, expressed as g digoxin/g tissue, was calculated. Thus, the amount of ^{3}H-digoxin present in the extracellular space was calculated and subsequently subtracted from the total radioactivity, the difference being identical with the cellular radioactivity. According to our determinations the extracellular space of guinea-pig atrial tissue is 0.35 ml./g (Lüllmann & van Zwieten, 1967). During contracture (induced upon incubation in 2,4-dinitrophenol) this parameter was reduced to 0.26 ml./g tissue. Division of the cellular radioactivity through the specific activity of the bath fluid yielded the amount of digoxin ($g \times 10^{-7}$ digoxin/g) taken up by the atrial tissue. If the atria were in contracture, owing to the treatment with high concentrations of digoxin, the reduced value of extracellular space (0.26 ml./g) was used for the calculations. The cellular uptake of digoxin thus calculated was plotted against the incubation time, for the six different concentrations studied (Fig. 1). This figure clearly demonstrates that at higher concentrations less time is required until the accumulation process reaches equilibrium. The

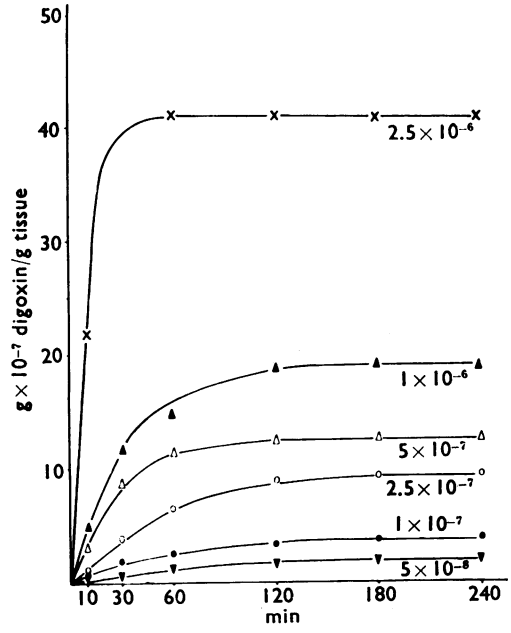

Fig. 1. Cellular uptake of digoxin ($g \times 10^{-7}$ digoxin/g tissue) by isolated atria plotted against incubation time in min, for six different concentrations of digoxin. Each point in this figure represents the mean of at least eight atria. S.E.M. about 5% for all points. The accompanying numbers of the various curves are indicating the incubation concentration (in g/ml.).

cellular uptake at equilibrium, divided through the digoxin concentration of the bath fluid yields the so-called tissue clearance—that is, the volume of the incubation medium, which contains as much digoxin as has been taken up by 1 g of tissue (Paton & Rang, 1965). Similar to the tissue/medium radioactivity ratio, the clearance also decreased when the medium concentration of digoxin was raised (Table 2).

The various amounts of digoxin taken up by the tissues were expressed as a function of the uptake at equilibrium, using the following equation:

$Y = A\ (1-e^{-kt})$, where Y is the uptake at time t and A the equilibrium uptake. The rate constant of uptake k may be obtained by plotting t versus log $\frac{A}{A-Y}$. The slopes of the straight lines thus obtained are representing the rate constants k. The values for k at the various concentrations studied are listed in Table 2. When the digoxin concentration of the medium is raised k also tends to increase, although it is doubtful whether this increase of k is significant. At the highest concentration investigated (2.5×10^{-6} g/ml.), however, k is considerably higher than for lower bath concentrations.

Plotting of the cellular uptake at equilibrium against the bath concentration did not yield a linear relationship (Fig. 2). Analysis of the curve represented in Fig. 2 indicates that there may be two different components to the digoxin uptake system. Subtraction of the linear component from the original curve yields a linear component. The latter is saturated at a bath concentration of approximately 5×10^{-7} g digoxin/ml. The significance of these findings will be dealt with in the discussion of this paper.

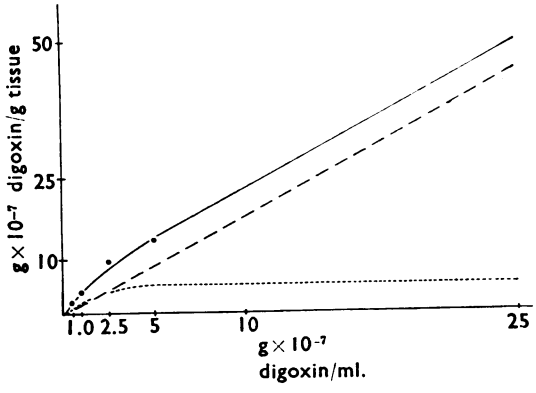

Fig. 2. Relationship between the cellular uptake at equilibrium and the concentration of digoxin in the incubation medium. Uptake in $g \times 10^{-7}$ digoxin/g tissue, concentration of the bath fluid in $g \times 10^{-8}$ digoxin/ml. The straight part of the graph (between 5×10^{-7} and 2.5×10^{-6} g/ml.) has been fitted through the experimental points by means of the method of the least squares. Equation of the regression line: $Y = 1.793x + 0.643$ ($Sy = 0.250$). The experimental points each represent the mean value of one series of isolated atria (8–10 organs per series). The dotted lines represent the two possible components of which the original curve may be composed. Subtraction of the linear component from the original curve yields the saturating component (see text).

The rate of uptake in the initial rapid phase was calculated from the steeply rising parts of the curves in Fig. 1. A straight line was fitted through the first three points on the curves by means of the method of the least squares. The slope of this line then represents the rate of uptake in the initial phase. Contracture was not observed for any of the concentrations within the period for which the straight lines were calculated. The calculated uptake rates enumerated in Table 2 showed linear dependency upon the five lowest concentrations, whereas at the highest concentration investigated (2.5×10^{-6} g/ml.) a higher rate of uptake occurred than would be in accordance with a linear relationship to the concentration. Accordingly, saturation of the uptake rate upon increasing medium concentrations did not occur for the range of digoxin levels studied

in our experiments. Owing to the relatively rapid development of contracture at the highest digoxin concentration studied, we decided not to investigate the uptake at still higher digoxin level of the bath fluid.

Influence of the frequency of beating on tissue accumulation of ^{3}H-digoxin

The frequency of concentration influenced the uptake of ^{3}H-digoxin by heart muscle tissue. Isolated left auricles were incubated as usual in a medium, containing ^{3}H-digoxin in a given concentration (1×10^{-7} g/ml.). The frequency was altered upon adaptation of the electrical stimulation. The cellular uptake of digoxin was expressed as g digoxin/g tissue upon calculation of the measured tissue radioactivity, as described above.

Resting left auricles took up the labelled glycoside more slowly than those beating 30 or 180 times/min, although the maximum uptake at equilibrium was approximately the same (Fig. 3). The $t_1/_2$ values of these uptake processes demonstrate the slower occurring uptake by resting auricles (resting auricles: $t_1/_2 = 52$ min; auricles beating either 30 or 180/min: $t_1/_2 = 28$ min). The differences in the amount of digoxin uptake between auricles beating at 30 and at 180/min, respectively, were not significant. The tissue clearances were not significantly affected by alterations of the frequency of contraction. Obviously the frequency rather influences the rate of uptake than the amount of digoxin accumulated.

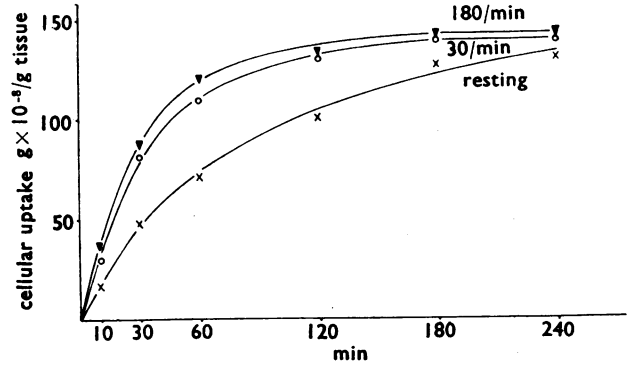

Fig. 3. Cellular uptake of digoxin by isolated left auricles, for three different frequencies of beating, plotted against incubation time (min). Digoxin concentration in the bath fluid: 1×10^{-7} g digoxin/ml. As in Fig. 1, each point on the curves represents the mean of at least eight atria. S.E.M. about 5%.

Influence of Ca^{2+} ions on uptake of ^{3}H-digoxin

The calcium content of the medium is known to influence the positive inotropic effect of cardiac glycosides (see summary by Klaus, 1964). It therefore seemed of interest to study the uptake of ^{3}H-digoxin at different calcium concentrations in the bath fluid. The accumulation of digoxin by isolated atria was established at three different calcium concentrations in the medium—that is, 0.36, 1.2 and 6.0 m.-equiv. Ca^{2+}/l., respectively. The given digoxin concentration was 1×10^{-7} g/ml. At this concentration digoxin exerts a moderate positive inotropic effect (Table 1). Apart from the variation of the calcium

content, the Tyrode solution was identical with those used in all other experiments. All our previous investigations had been carried out with a Tyrode solution, containing 1.2 m-equiv Ca^{2+}/l. Increasing of the calcium level to 6.0 m-equiv/l. neither affected the amount of digoxin accumulated, nor the rate of uptake (Fig. 4) ($t_1/_2$ about 36 min). If, however, the calcium concentration was reduced to 0.36 m-equiv/l. the accumulation of digoxin took place considerably slower ($t_1/_2$ about 63 min) than at a calcium level of 1.2 m-equiv or 6 m-equiv/l. Still, after longer periods of incubation, the tissue uptake at 0.36 m-equiv/l. achieved had approximately the same order of magnitude as that at the usual calcium level.

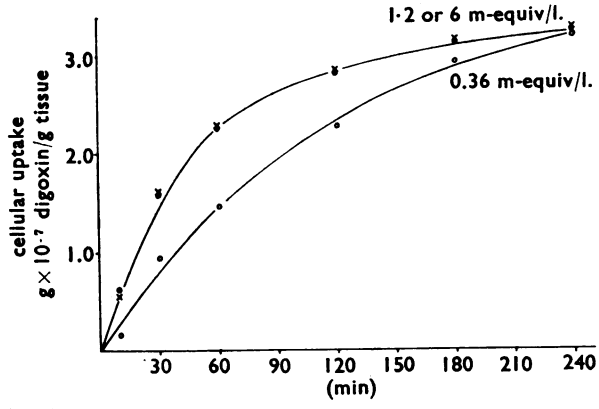

Fig. 4. Uptake of digoxin by isolated atria at different calcium levels of the bath fluid. Cellular uptake ($g \times 10^{-7}$ digoxin/g tissue) plotted against incubation time (min). Digoxin concentration of the medium: 1×10^{-7} g/ml. The uptake curves at 1.2 m-equiv, Ca^{2+}/l. (symbol ×) and the one at 6 m-equiv/l. (symbol ●) coincide. The uptake process at a Ca^{2+} level of 0.36 m-equiv/l. occurs considerably more slowly. Each point on the curves represents the mean of about eight atria. S.E.M. approximately 5%.

As could be expected, in mechanograms the contraction amplitude of the atria was considerably reduced in a medium containing as little as 0.36 m-equiv calcium/l. The frequency of beating remained unaffected. However, the onset of the positive inotropic effect of digoxin (2.5×10^{-7} g/ml.) was delayed from 5 to 18 min.

Release of ^{3}H-digoxin

The release of ^{3}H-digoxin, previously accumulated in isolated atria, occurred relatively slowly. The decrease of the cellular digoxin concentration was plotted against the time of incubation in the inactive, digoxin-free tyrode solution (Fig. 5). The loss of digoxin took place more rapidly at a high initial concentration. A relatively smaller amount of digoxin was left in the tissue when the original digoxin level was high. The curves represented in Fig. 5 were plotted in a semilogarithmic system.

Straight lines were not obtained. After initial incubation in a medium, containing 2.5×10^{-6} g digoxin/ml. it took about 50 min before 50% of the accumulated digoxin had been lost, whereas after a wash-out period of 3 hr 23% of the initial amount had been released. For the wash-out process after incubation in 5×10^{-7} g/ml. the corresponding figures were 70 min and 34%, and for the lowest concentration (1×10^{-7} g/ml.) 155 min and 47%, respectively.

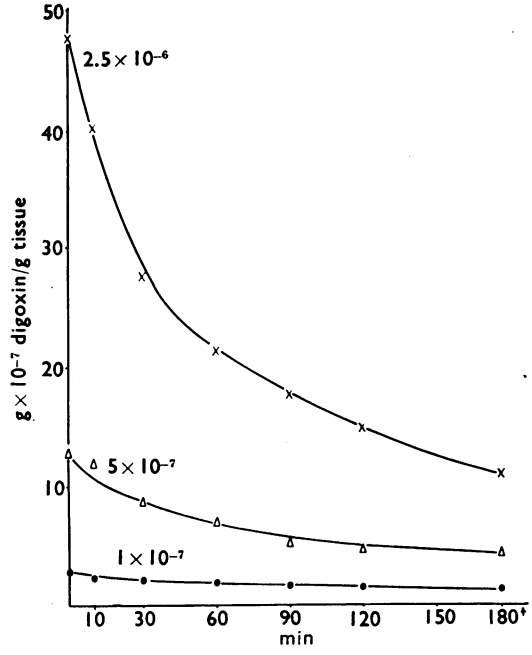

Fig. 5. Release of previously bound digoxin upon incubation of the atria in digoxin-free Tyrode. Digoxin concentration of the atria ($g \times 10^{-7}$ digoxin/g tissue) plotted against incubation time (min) in the digoxin-free Tyrode solution. Before the release process, the atria have been incubated in three media with different digoxin concentrations (2.5×10^{-6}, 5×10^{-7} and 1×10^{-7} g/ml.). For details see text. As in all other figures, the points on the curves are representing the mean values of at least eight atria ; S.E.M. about 5%.

DISCUSSION

The results described in the present paper demonstrate that the uptake of ^{3}H-digoxin by guinea-pig isolated atria occurs rather slowly, especially when the medium concentration of digoxin is low. Only upon incubation in relatively high concentrations of digoxin, the uptake process reaches equilibrium within 1 hr. For the lowest concentrations 2–3 hr are required until the stage of equilibrium has been reached. The uptake of catecholamines by the isolated perfused rat heart (Iversen, 1963 ; 1965a, 1965b) and that of prilocaine by guinea-pig isolated atria (Lahrtz, Lüllmann & van Zwieten, 1967), for instance, are taking place considerably more rapidly. From clinical experience it is known that the therapeutic action of digoxin begins but after a few hours. Possibly this period of latency might be related in some way to the digoxin uptake process in the heart.

According to Fig. 1, the tissue accumulation is accelerated upon increasing the digoxin concentration of the medium, which is reflected by the $t_1/_2$ values listed in Table 2. It remains doubtful, however, whether the differences between the $t_1/_2$ values are significant. The rate constants k (Table 2) also show a tendency to increase when the digoxin concentration is raised. The increase of k is probably not significant, although at the

highest concentration investigated the rate constant is much higher than those calculated for the lower medium concentrations.

It should be noted, however, that this highest concentration (2.5×10^{-6} g/ml.) is already a toxic one: the isolated atria readily developed contracture. This toxic process might also influence the rate constant of digoxin uptake. Since the uptake rate does not decrease within the range of concentrations studied in our experiments, it cannot be established whether the digoxin uptake follows Michaelis-Menten kinetics or not. At the highest concentration investigated (2.5×10^{-6} g/ml.) contracture of the isolated organ easily developed. Therefore, we felt that the investigation of still higher digoxin concentrations would give rise to a rather unphysiological situation which would not be of particular significance. The cellular clearance achieved by isolated atria for digoxin proved to be in the range 2–4, depending on the medium concentration. These figures were rather low, compared to the uptake of catecholamines by isolated rat heart for instance (Iversen, 1963, 1965a, 1965b). The presence of special stores for catecholamines in the heart explains the tissue's high uptake capacity for these neurohormones. Of course, no such stores exist for digoxin.

No linear relationship was obtained when the cellular uptake at equilibrium was plotted against the medium concentration (Fig. 2). Analysis of this curve showed that it may be the sum of two different components. The linear component which is not subject to saturation possibly represents passive or leak moiety of the digoxin across the membrane. The other component which shows saturation at a medium concentration of about 5×10^{-7} g digoxin/ml. probably represents the accumulation process as such. For this accumulation (or adsorption) it might be expected that such a process is liable to saturation. The high clearances at the lower bath concentrations (Table 2) and also the slow release at lowest concentration (Fig. 5) would be in agreement with a saturation phenomenon. Half-saturation occurs at approximately 2.5×10^{-7} g/ml. It might be interesting to emphasize that *in vitro* 50% inhibition of cardiac Na-K-ATPase occurs at glycoside concentrations within the same order of magnitude. Moreover, *in vivo* this concentration also corresponds to a therapeutic glycoside level (literature, see Klaus, 1964). In studies on the distribution of digitoxin in the rat, no particularly high concentration of this cardiac glycoside could be detected in the heart (Repke, 1958a, 1958b). Since the rat is very insensitive to the action of cardiac glycosides, it remains doubtful whether conclusions from such experiments in this animal species may be generalized (Dutta *et al.*, 1963). However, recent investigations by Marcus *et al.* (1966) on the fate of intravenously injected ^{3}H-digoxin in the dog have indicated that also in this species a specific accumulation in the heart does not occur. According to these investigations the radioactivity in heart muscle tissue was about three times higher than that in blood plasma. In the kidney and also in the colon the ^{3}H-digoxin content was considerably more elevated. Consequently, the uptake capacity of the latter two organs seems to be higher than that of the organ in which the cardiac glycoside exerts its characteristic therapeutic action.

Also in man, left auricles were shown to accumulate but relatively little of an injected amount of ^{3}H-ouabain (Marks *et al.*, 1964). Autoradiographic studies have demonstrated that tritium labelled digitoxin is chiefly bound in the so-called A-band and at the cell membranes in heart muscle tissue (Conrad & Baxter, 1964). The relationship

between these autoradiographic studies and our kinetic investigations could not be established so far.

Although in our experiments digoxin uptake was not affected by a reduction of the frequency of beating from 180 to 30/min, resting left auricles took up ^{3}H-digoxin considerably more slowly than beating organs. Consequently, the uptake process seems to be related in some way to excitation phenomena at the membrane—that is, the occurrence of action potentials or the contraction as such facilitates the accumulation of ^{3}H-digoxin. The positive inotropic effect of cardiac glycosides is known to be dependent on the relationship between the various calcium fractions within the heart muscle cell (Klaus & Lüllmann, 1964). Moreover, calcium ions in the medium and cardiac glycosides are known to enhance each other's positive iontropic action—that is, they act synergistically (see Klaus, 1964). In our experiments, lowering of the calcium level of the Tyrode solution from 1.2 to 0.36 m-equiv/l. did not affect the relative magnitude of the positive inotropic effect of a given concentration of digoxin. The onset of this effect, however, proved markedly delayed in a Tyrode solution with low calcium content (0.36 m-equiv/l.). The experiments with the tritium labelled digoxin have pointed out that the uptake of the drug occurs also considerably more slowly at this low calcium level in the medium. After a longer period of incubation (about 3 hr) the amount of glycoside taken up was approximately the same as that accumulated at the higher calcium level (1.2 m-equiv/l.). The uptake process was not affected by increasing the calcium level in the medium from 1.2 until 6 m-equiv/l. The positive inotropic effect remained unchanged either. Thus the uptake process seems to be related, in a so far unknown way, to the positive inotropic effect of the drug involved.

As could be expected from the uptake experiments, the release of previously bound ^{3}H-digoxin also takes place rather slowly. At higher tissue concentrations the digoxin is washed out more rapidly. This finding could be considered more or less analogous to those obtained for the uptake at various medium concentrations. The slowly occurring release process would indicate that the drug is bound relatively firmly to the tissue. It has already been known for some time that the clinical actions of digoxin in patients are relatively long-lasting. Marcus *et al.* (1966) have demonstrated that in the anaesthetized dog ^{3}H-digoxin is but slowly released by the various organs which have accumulated the drug after intravenous administration. Thus the slow release of ^{3}H-digoxin, observed in our studies on isolated organs, is not conflicting with previously obtained experimental and clinical findings.

Various studies have confirmed that digoxin is not easily metabolized, either *in vivo* or *in vitro* (Lage & Spratt, 1965 ; Marcus *et al.*, 1964). According to these authors, *in vivo* degradation of the cardiac glycoside takes place to an appreciable extent only in the liver. Also *in vitro* digoxin is metabolized by liver slices but not by heart muscle slices or homogenates (Lage & Spratt, 1965). Our observations that ^{3}H-digoxin remained unaltered in the guinea-pig isolated atria used in our studies were therefore not surprising. The digoxin preparation used had been obtained by means of the Wilzbach method. According to Abel *et al.* (1965), such a radioactive digoxin preparation is fairly stable and does not give rise to a significant degree of hydrogen exchange, both *in vitro* and *in vivo*. In our studies, which have been carried out with the same type of radioactive preparation, we could not find any chromatographic evidence for such an exchange phenomenon.

SUMMARY

1. The accumulation and release of ^{3}H-digoxin by electrically stimulated guinea-pig isolated atria (or left auricles) has been studied under various experimental circumstances.

2. The uptake has been determined for six different bath concentrations of digoxin, in the range 5×10^{-8} until 2.5×10^{-6} g/ml. The lowest concentration does not influence the contraction amplitude of the atria, whereas the highest concentration has a marked positive inotropic effect and provokes rapid development of contracture. The accumulation of digoxin takes place more rapidly and sooner reaches equilibrium when the medium concentration is raised. Depending on the concentration, equilibrium is reached after 1–3 hr incubation. The tissue/medium radioactivity ratios, which lie within the range 1.5–3, are reduced when the medium concentration is increased.

3. Resting left auricles accumulate ^{3}H-digoxin more slowly than beating organs. After 3 hr the amount of digoxin taken up by resting auricles is approximately the same as that accumulated by beating organs. Reduction of the frequency of beating from 180 to 30/min does not affect the uptake process.

4. Reduction of the calcium content of the bath fluid from 1.2 to 0.36 m-equiv/l. provokes a slower uptake of digoxin, although after 3 hr incubation the amount taken up is the same at both calcium concentrations. The lower calcium level of the bath fluid leads to a considerable delay in the onset of the positive inotropic effect of digoxin. This delay might be somehow related to the slower uptake of the drug. An increased calcium level of the medium (6 instead of 1.2 m-equiv/l.) neither affects the accumulation of digoxin nor its positive inotropic action.

5. The release of previously bound ^{3}H-digoxin is influenced by the initial tissue concentration of digoxin. At higher concentrations a relatively larger amount of digoxin is washed out and the release process takes place more rapidly than at lower tissue concentrations of the drug.

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