Biochemical Pharmacology, Vol. 16, pp. 1165–1174. Pergamon Press Ltd. 1967. Printed in Great Britain

THE UPTAKE AND CONTENT OF DIGITOXIN AND ITS METABOLITES IN THE HEART MUSCLE OF RATS AND GUINEA-PIGS AFTER ACUTE AND CHRONIC APPLICATION*

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(*Received* 24 *November* 1966; *accepted* 23 *January* 1967)

Abstract—On the basis of the xanthydrol reaction and a highly sensitive thin-film chromatographic detection method, content and composition of glycoside metabolites were determined in the heart muscle and in the blood of rats and guinea-pigs after single and repeated i.p. injections of digitoxin. In the guinea-pig's heart we found a maximum of 1 μg/g, in the rat's heart 7–8 μg glycoside/g heart muscle. A pretreatment by reserpine did not influence the uptake of digitoxin. Digitoxin metabolites, inclusive of genins, were found in the heart muscle most frequently 14–24 hr after application of digitoxin, C_{12}-hydroxylation products 6 hr at the earliest after injection. The proportion of metabolites was always relatively small in comparison with unchanged digitoxin. After subacute digitoxin injection the proportion of digitoxin in the heart muscle was much bigger than after only one injection. If the content of digitoxin and its metabolites was compared in the blood and in the heart muscle, a correlation with the digitoxigenin derivatives would be seen. The digoxigenin derivatives, almost without exception, were more frequently detected in the blood rather than in the heart muscle.

IN THE past 20 yr the fate of digitoxin in animal and human organisms has been investigated first of all by using more sensitive biological[34, 16, 28] and isotopic methods[31–33] but in the last 10 yr it has been re-investigated by using modern chemical detection methods, too[1, 37, 41, 25, 26, 20, 55]. Several authors have unanimously found out that there was no preferential linkage of glycoside to the heart.[3, 12, 17, 40, 42, 33] While there is much information available about the amount of glycoside uptake by the relatively insensitive heart of the rat by isotopic methods, and even more information by chemical detection methods,[3, 12, 38, 42, 46] similar investigations in digitalis-sensitive animal species, such as cats and guinea-pigs, and in human beings have been carried out only by adopting isotopic methods, due to the difficulties which had to be overcome in the detection of glycoside.[12, 28, 46] In addition, little has been done so far[12] to find out whether the uptake of glycoside by the heart is affected under various physiological and pharmacological test conditions. Several authors have detected a diminished glycoside toxicity and a weaker positive isotropic glycoside

* Compiled on the basis of papers read out at the 6th and 7th meeting of the work-group of industrial and college pharmacologists of the G.D.R. held in Erfurt (1964) and Weimar (1965).

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effect after depletion of amine through reserpine or guanethidine,[35, 36, 49, 50, 51, 11, 6, 7, 54, 13, 15] and it has remained unknown, whether reserpine—similar to the biogene amines—reacted through offsetting its binding capacity or the glycoside uptake resp., or whether the diminished content of pyrocatechol had induced the weakening of the glycoside effect.

Brown, Wright and Okita[5] were the first to have detected the presence of C_{12}-hydroxylation products of digitoxin in the urine and, soon afterwards, Repke[40] proved that they were in the heart,[38] the urine and the faeces[39] and in the bile[10] of rats, too. The share of these hydroxylation products was reported to increase continuously from 23 to 56 per cent between 6–48 hr after i.v. injection of digitoxin,[43] so that the digitoxin derivatives would assume therapeutical importance upon the digitoxin effect.[38] Though in other papers the metabolites formed *in vivo* from digitoxin have been described in detail and e.g. the digoxigenin-*bis*-digitoxoside could be identified as the major secretion product of digitoxin (62 per cent),[45] a differentiation and quantitative analysis of the individual metabolites in the heart muscle was unsuccessful. Clarification of this question, however, would be very important from the therapeutical standpoint, since only those metabolites traceable in the heart muscle are responsible for the clinical effect of digitoxin. Should after 24–48 hr actually more than 50 per cent of the heart glycoside content be constituted of C_{12}-hydroxylation products, the digitoxin effect would strongly approach the digoxin effect.

It was the aim of this paper to determine the extent of digitoxin uptake and the period of digitoxin linkage in the heart of rats and guinea-pigs in a comparative way, and to find out whether depletion of pyrocatecholamine would affect the uptake of glycoside due to preliminary treatment by reserpine. In addition, a differentiation of the glycoside bound in the heart muscle should be achieved and semi-quantitative estimates of the individual metabolites should be made. Apart from this, the question of glycoside distribution between blood and heart muscle should be investigated. According to Repke[40] a uniform and constant relationship between the blood level and the corresponding tissue concentration of digitoxin should prevail up to 12 hr after i.v. injection. From these findings it appeared to be justified to predict conclusively the glycoside content in the heart muscle on the basis of the serum concentration of a digitalis body.[30] A deeper insight into the dynamics of metabolite distribution between serum and heart muscle should be obtained by differentiating the individual metabolites, something about which little is so far known.

METHODS

By modifying the procedure described by Repke[41] for the quantitative detection of glycoside in the heart of rats and guinea-pigs, we used always 20 Wistar-rats of both sexes with a weight of about 100–150 g and each 40 guinea-pigs of both sexes and different origin with a weight of about 300–350 g. The rats were given i.p. 5 mg/kg, the guinea-pigs 1·5 mg/kg paperchromatographically pure digitoxin* freshly resolved in 1% pyridine and 25% alcohol solution. The rats were killed after 5, 15, 180 min, 5 1/2, 7, 8, 14 and 24 hr, and the guinea-pigs

* We would like to thank Dr. Baumgarten, VEB Ysat, Wernigerode, who undertook to produce paper chromatographically pure digitoxin and placed it at our disposal.

after 5, 15, 30, 60, 120, 180 min and 8 hr after injection of digitoxin, the hearts were homogenized, and the resultant homogenate—as previously indicated—freed from residual fat by petroleum ether and carbon tetrachloride and four times extracted by using double the amount of chloroform. After the evaporation of chloroform the residue was absorbed by 10% alcohol in chloroform, and cast on the aluminium oxide column pretreated with methylene chloride. Based on the xanthydrol reaction[37] the portions elutriated by methylene chloride and increasing quantities of methanol were tested for their glycoside content, but only elutriate 4 and 5 contained glycosides (4th elutriate with 0·6 ml CH_2Cl_2 and 4·4 ml CH_3OH and the 5th elutriate with 2 ml CH_2Cl_2 and 3 ml CH_3OH). Eight test series with guinea-pigs and 13 test series with rats were carried out in all. In control tests after addition of digitoxin and its metabolites we recovered 85–88 per cent of the glycosides added. The sure detection limit was about 5 μg of glycoside/heart homogenate when using the xanthydrol reaction.

In the tests for the qualitative differentiation of digitoxin and its decomposition products in the heart of rats, paperchromatographically pure digitoxin, freshly resolved in 40% ethanol, was administered i.p. to Wistar rats of both sexes having a weight of about 100–150 g. Maximally compatible doses of 5–10 mg/kg were injected according to the period of exposure. After a single exposure the test periods were varied from 3–92 hr. In subacute and chronic tests digitoxin was administered in maximally compatible doses of 2–3 mg/kg/day for 4–79 days, in order to simulate the conditions of a long-term clinical application of digitoxin. For each test the blood and the heart muscle of each 20 rats were investigated. Thirty-five test series were carried out in all. The chemical detection methodology of digitoxin and its metabolites was described in detail at a different place.[19] The glycoside quantities on the thin-film chromatograms were assessed semi-quantitatively with + to ++++ according to the size of stains and intensity of fluorescence. In the graphs the share of the individual metabolites in all tests within a test period and one dosage was converted into percentage and represented in a graphical manner.

RESULTS

1. *Uptake of glycoside in the heart muscle of rats and guinea-pigs without and with a preliminary treatment by reserpine*

The results of reserpine-non-treated rats and guinea-pigs are summarized in Fig. 1. It can be taken from this graph that already 5 min after injection a maximum

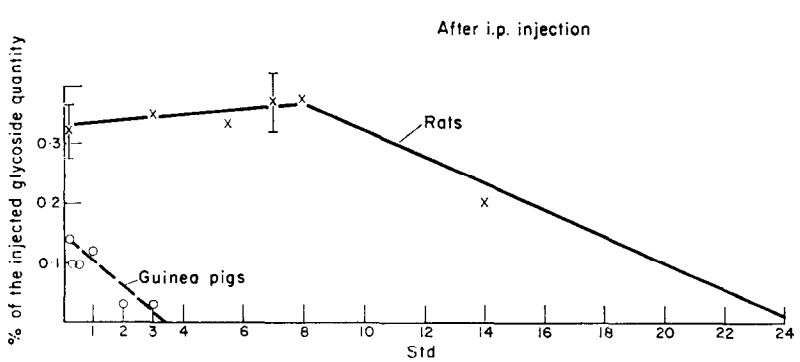

FIG. 1. Digitoxin content in the heart.

concentration of glycoside was reached in the heart of rats and guinea-pigs. Calculated on the administered glycoside quantities we found that a maximum of 0·3–0·4 per cent was contained in the hearts of rats, but in the hearts of guinea-pigs only about 0·1 per cent. The difference between the species of rats and guinea-pigs becomes even more conspicuous, when the conversion of the glycoside quantities bound by the heart is expressed in g/heart weight (moist weight) instead of in percentage of the injected dose. Five minutes after i.p. injection a maximum of 1 μg/g was found in the heart of guinea-pigs, in the rat hearts, however, a maximum of 7–8 μg/g digitoxin. In non-conformity with the findings by Repke[42] the digitoxin concentration in rat hearts remained unchanged up to 8 hr. After 14 hr, however, the glycoside content decreased considerably and 24–48 hr after application it sank below the detection limit set by the xanthydrol reaction. In contrast, digitoxin was bound by the guinea-pig heart only for 1–2 hr, and after 3 hr low amounts of glycoside were detectable. After a preliminary treatment with 10 mg/kg of reserpine, the uptake of glycoside did not change for 24 hr in comparison with control tests carried out without reserpine application. In both test series 0·32 per cent of the injected total amount of glycoside was found.

2. *Detection and differentiation of the digitoxin metabolites in the heart muscle and blood of rats after one and repeated injections of digitoxin*

The results after an addition of digitoxin are found in Fig. 2. It can be seen from the graph that metabolites were most frequently found 14–24 hr after injection of digitoxin. Within this period all the decomposition products of digitoxin—only traces of

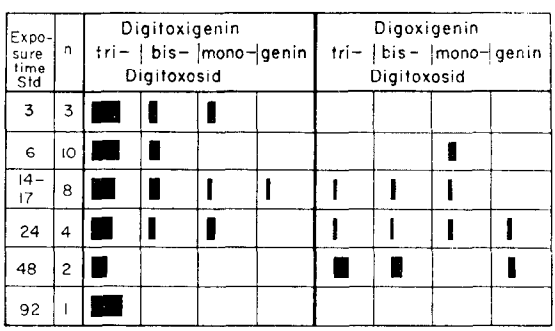

FIG. 2. Digitoxin metabolites in rats' heart after one digitoxin injection.

them in most cases—were detectable. The share of the individual derivatives was low compared with the content of unchanged digitoxin. After injection of digitoxin small amounts of C_{12}-hydroxylation products occurred in the heart muscle 6 hr at the earliest following the injection; 48 hr after digitoxin addition, they reached a maximum share of about 50 per cent, but 92 hr after administration of digitoxin they were completely absent, although at this time digitoxin was still traceable in the heart muscle. In contrast to Repke[43] we found even genins, digitoxigenin and digoxigenin, in the heart muscle, but no 3-epi genins.

The results of subacute and chronic tests (Fig. 3) have essentially confirmed the findings obtained after a non-recurring administration of digitoxin. The overwhelming

share of digitoxin in the heart muscle became more strikingly apparent in subacute tests rather than in acute tests. A genin was found once in two test series, i.e. digoxigenin. Besides, we detected digitoxigenin and digoxigenin-*bis*-digitoxoside. In the chronic tests, 6 hr after the last digitoxin injection and a 79-day pretreatment, digitoxigenin-*bis*-digitoxoside was detectable in greater quantities in the heart muscle than digitoxin and digoxigenin-*bis*-digitoxoside, whereas in a second test series, 17 hr after the last digitoxin injection and a 63-day test period, digoxigenin-*bis*-digitoxoside surpassed the share of the digitoxin in the heart.

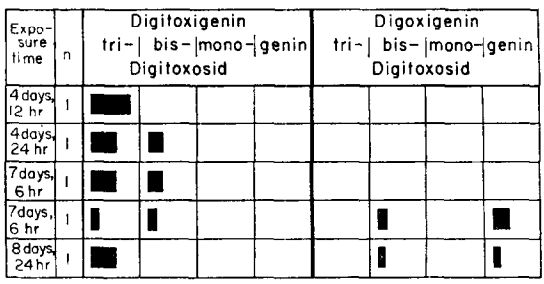

FIG. 3. Digitoxin metabolites in rats' heart after subacute injection.

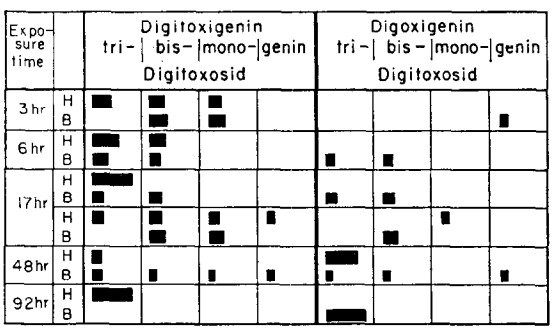

FIG. 4. Digitoxin metabolites in the heart and in the blood of rats after one injection.

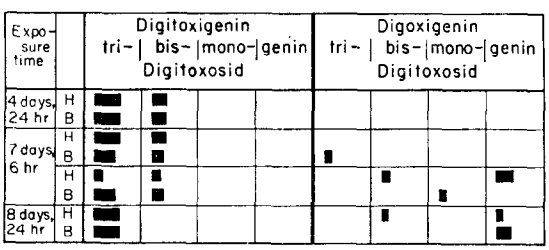

FIG. 5. Digitoxin metabolites after subacute administration.

Comparing the presence and the quantity of digitoxin metabolites in the blood and in the heart muscle, you will find—in conformity with the tests for one (Fig. 4) and a repeated (Fig. 5) application of digitoxin—a certain concurrence in the share of metabolites in the digitoxin derivatives, although this concurrence was by and large

absent at later periods of observation (after 48 and 92 hr). This concurrence was absent almost without exception in the acute and subacute tests with digoxigenin derivatives which were more frequently detectable in the blood rather than in the heart muscle. We found digitoxigenin and digoxigenin in the blood during three tests in all.

DISCUSSION

Among the conprehensive literature about digitalis there are only a few papers which are concerned with the mechanism of glycoside uptake and the metabolic processes of digitalis bodies in the heart muscle. The reason for this might be ascribed to the difficulties encountered in the detection of minute amounts of glycoside in biological tissues. The frequently used xanthydrol reaction is relatively insensitive and does not permit a differentiation of the digitalis metabolites. For the chemical detection of digitalis rats, which have a heart muscle that is relatively insensitive to glycosides, have been used so far as test animals. The present tests carried out with guinea-pigs have shown that the xanthydrol reaction has also been suitable for the detection of digitoxin metabolites in the heart muscle of digitalis-sensitive animal species, when using adequately large groups of animals.

A generally accepted explanation of the low digitalis sensitivity of the rat's heart has not been given so far. In the present paper a comprehensive investigation was made of the amounts of glycoside which the heart muscle of rats and guinea-pigs were able to bind, and it was possible to exclude the fact that the differing sensitivity of the two species of animal was due to quantitative differences in the binding capacity for digitalis bodies. The digitalis-sensitive guinea-pigs heart—according to our findings—was apt to bind *in vivo* only about 1/8 of the glycoside amount g/heart tissue that can be bound by the heart muscle of the rat. Sjoerdsma and co-workers,[46] however, have found out that there was no essential difference between the uptake by the isolated rat and guinea-pig heart muscles, but between that of rats and cats. In addition, the heart glycosides remained in the heart of guinea-pigs only for some 1–2 hr, whereas they were detectable at least for 14 hr in the heart muscle of the rat by adopting the xanthydrol reaction. Later experiments carried out on the basis of the more sensitive thin-film chromatographic method have shown that digitoxin was detectable for more than 92 hr after only one injection, so that digitoxin and its metabolites would be traceable presumably even in the guinea-pig heart for more than 3 hr, if sufficiently sensitive detection methods were available. Nevertheless, the relationship of the linkage period obtained from the tests on the xanthydrol reaction might be characteristic of both the animal species. Therefore, it is impossible to explain the deviatingly low sensitivity of the rat's heart to digitalis bodies by a smaller or short-term glycoside binding in the heart muscle. The shorter period of a digitoxin linkage by the guinea-pig heart is well in conformity with the experience gathered in pharmacological experiments[23, 18] and with the high rate of depoisoning,[22] and it possibly accounts for the inactivity of digitoxin following oral and intraduodenal application.[24, 14] It would be thinkable that the rate of resorption from the gastro-intestinal tract did not essentially exceed the period of linkage to the heart muscle, so that a higher tissue glycoside level could not be attained. Further investigations must show whether this working hypothesis will prove to be correct, since glycosides with a medium persistency (e.g. gitoxin derivatives, digoxin) become effective in guinea-pigs even after oral or intraduodenal administration, so that it

must be assumed implicitly that they are either bound to the heart muscle over a longer period or secreted more slowly. Despite several studies by Repke[43] and Lauterbach[27] too little is still known about the relationship of the resorption factor, the linkage period to the heart, the period of the enterohepatic circulation and the rate of glycoside decomposition and glycoside secretion, in order to be able to give a substantiated explanation of the different oral activities and the different persistencies of individual heart glycosides for the various animal species.

Beginning with the known amine-depleting effect by reserpine and in connection with numerous bibliographical data on a diminished toxic and therapeutical digitalis effect after reserpine pretreatment, it was reasonable to decide the glycoside content in the heart muscle in a comparative quantitative manner without and with a reserpine pretreatment. The diminished glycoside uptake could serve as an obvious explanation of the different effects due to reserpine pretreatment. This hypothesis, however, could not be reaffirmed by the present investigations. Reserpine, after one administration of maximally compatible doses, had no detectable influence on the digitoxin content in the heart. Moreover, its fibrillation-adverse effect on the heart muscle might lead to an inhibition of the intoxication phenomena detectable by the electrocardiogram, thus increasing the lethal dose.

The preparation of a highly sensitive thin-film chromatographic detection methodology for digitoxin and its metabolites made it possible for the first time to observe the changes in the heart with regard to the content of individual metabolites at different times after one and repeated additions. Our findings have supported the fact that in rats after one or repeated additions unchanged digitoxin and digitoxigenin metabolites resulting from digitoxin constitute the major amount of all glycoside bound by the heart. In individual tests we have even found a bigger share of C_{12}-hydroxylation products in the heart. During both the tests carried out in a 48-hr exposure to digitoxin, the digoxigenin substances were predominant. After 92 hr digitoxin alone was detectable in the heart muscle. These results indicated the fact that the extent of C_{12}-hydroxylation fluctuated from test to test, and after equal test periods not always equal biological effects could be expected. It is known even from other enzymatic reactions that the rate of their sequel *in vivo* is subject to big fluctuations. It is obvious from the tests, too that digitoxin metabolites occur in small quantities in the heart after 3 hr, digoxigenin-compounds after 6 hr at the earliest, but mostly after 14 hr. In the comparative determination of the digitoxigenin and digoxigenin-compounds in the blood and in the heart muscle it became furthermore obvious that digoxgenin derivatives were more frequently detectable in the blood than in the heart muscle. All these findings could well be supported by the assumption that the heart muscle already a few minutes after digitoxin injection provided its receptors with unchanged digitoxin which showed a powerful and protracted adhesive strength. Excess digitoxin circulating in the blood was decomposed in the periphery (liver), so that all the metabolites would become discoverable one beside the other in the blood. These, however, could be bound to the heart muscle only in such measure, as part of the adhesive digitoxin was released or decomposed. Therefore, it appears to be understandable that C_{12}-hydroxylation products exceed in the blood, and that no complete equilibrium is achieved between the metabolite content in the blood and the heart muscle. In the same measure as the excess digitoxin which is never bound to the heart is subject to decomposition and secretion, the digitoxin metabolites, after

only one digitoxin application, will largely disappear from the blood and the heart muscle, or be present only in such minute quantities that defy even our very sensitive detection method; for it is now clear that metabolites can only be formed from the heart-bound digitoxin and this process is known to proceed very slowly. Therefore, it was probably not accidental that 92 hr after digitoxin injection no metabolite could be found in the heart muscle, apart from digitoxin, though low amounts of digoxin were traceable in the blood. After sub-acute injection it is reasonable that the longest-adhesive glycoside, i.e. digitoxin and digitoxigenin-*bis*-digitoxoside, prevail in the heart still more strongly than after only one injection.

If it is allowed to generalize the present experiments with rats, it can be stated in a summary that the metabolites of digitoxin formed in the liver do not have any importance, or at most only a subordinate one, for the heart effect, after one, but more clearly after repeated injections. The result is well in keeping with clinical experience which permits one to distinctly differentiate the digitoxin effect from the digoxin effect. The chemical differentiation of the metabolites bound in the blood and in the heart muscle has furthermore confirmed the experience gathered by the pharmacologists and clinicians that the effective glycoside level cannot be identified with the glycoside blood level, and that the nature and quantity of the heart-bound glycoside cannot be inferred from the nature and the quantity of glycoside metabolites in the blood.

Particular reference has to be made to one finding, since it stands in contradiction to the data published by Brown and co-workers[4] and Repke.[42] In a greater number of experiments genins were detected (digitoxigenin and digoxigenin, but no 3-epi-genin) in the blood and even in the heart muscle. Since, in preparing the methods[19] the fact that during the extraction and cleaning phases no genins were formed *in vitro* from monosides was repeatedly checked, the divergence between our findings and those by Brown and Repke must be attributed to the lower sensitivity of the detection methods used by these authors. Repke,[43] however, after intragastric digitoxin injection was able to detect digitoxigenin in the stomach and in traces even in the duodenum, and Lauterbach[27] could detect it in the bile, 1 hr after intraduodenal digitoxin injection, which serves as an indication of the fact that genins are in general not so quickly metabolized *in vivo* to 3-epi-genins, as it was assumed by Repke due to his negative findings. With these results, however, the genin hypothesis of the digitalis effect can by no means be rigorously rejected, as suggested by Repke. Since the autoradiographically detectable localization of glycosides at the A-bands is in favour of a point of attack of the digitalis effect in the subcellular range of the endoplasmatic reticulum,[48, 9, 52, 47, 53, 21] where the specific effect is likely to be released already by minute quantities of digitalis, it is understandable that these low amounts of glycoside were able to withstand a less sensitive detection methodology. Since our results obtained on the basis of a more sensitive method refer to the possible presence of genins in the heart muscle and in the blood of rats, further experiments using other methods will enable a decision in favour or against the genin hypothesis.

REFERENCES

1. R. C. ANDERSON and K. K. CHEN, *J. Am. pharm. Ass.* (*Scient. Ed.*) **35**, 353 (1946) cit. according to WRIGHT, *The Metabolism of Cardiac Glycosides*. Blackwell, Oxford.
2. J. R. ASHLEY, B. T. BROWN, G. OKITA and S. E. WRIGHT, *J. biol. Chem.* **232**, 315 (1958).

3. R. BINE, M. FRIEDMAN, S. O. BYERS and C. BLAND, *Circulation* **4**, 105 (1951).
4. B. T. BROWN, E. E. SHEPPARD and S. E. WRIGHT, *J. Pharmacol.* **118**, 39 (1956).
5. B. T. BROWN, S. E. WRIGHT and G. T. OKITA, *Nature, Lond.* **180**, 607 (1957).
6. V. CAIROLI, J. REILLY, R. ITO and J. ROBERTS, *Fedn Proc.* **21**, 127 (1962).
7. V. CAIROLI, J. REILLY and J. ROBERTS, *Fedn Proc.* **20**, 122 (1961).
8. D. CHRISTOPH and W. FÖRSTER, incl. discussion by K. LOHMANN, *Verh. Ges. exp. Med.* 5th Conf., Steinkopff, Dresden (1964).
9. L. L. CONRAD and D. J. BAXTER, *J. Pharmacol.* **145**, 210 (1964).
10. E. COX and S. E. WRIGHT, *J. Pharmacol.* **126**, 117 (1959).
11. F. DENIS, A. CESSION-FOSSION and A. DRESSE, *C.r. Séanc. Soc. Biol.* **157**, 206 (1963).
12. C. S. FISCHER, A. SJOERDSMA and R. JOHNSON, *Circulation* **5**, 496 (1952).
13. W. FÖRSTER and U. STOLZENBURG, *Acta biol. med. germ.* **11**, 86 (1963).
14. W. FÖRSTER, I. GUHLKE, R. HERRMANN and G. KRÖMER, *Archs int. pharmacodyn. Thér.* **159**, 1 (1966).
15. W. FÖRSTER and S. WAGNER, non-published experiments.
16. M. FRIEDMAN and R. BINE, JR., *Proc. Soc. exp. Biol. Med.* **64**, 162 (1947).
17. M. ST. FRIEDMAN, S. GEORGE, R. BINE, S. O. BYERS and C. BLAND, *Circulation* **6**, 367 (1952).
18. H. GIERTZ, F. HAHN and R. SCHUNK, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **221**, 34 (1954).
19. K. GRADE and W. FÖRSTER, in cooperation with S. SCHULZECK, *Biochem. Pharmac.* in press.
20. G. GRIMMER, W. KÜSSNER and K. LINGNER, *Arzneimittel-Forsch.* **10**, 28 (1960).
21. S. C. HARVEY and G. R. PIEPER, *J. Pharmacol.* **114**, 14 (1955).
22. J. HERRMANN and K. REPKE, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **247**, 19 (1964).
23. G. HOFFMANN and L. LENDLE, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **212**, 376 (1951).
24. R. HOTOVY, *Arzneimittel-Forsch.* **1**, 160 (1950).
25. K. B. JENSEN, *Acta pharmac. tox.* **10**, 69 (1954).
26. K. B. JENSEN, *Acta pharmac. tox.* **9**, 66 (1953).
27. F. LAUTERBACH, in cooperation with D. NITZ and K. PRESCHER, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **247**, 391 (1964).
28. R. A. LEHMANN and G. H. PAFF, *J. Pharmacol.* **75**, 207 (1942).
29. B. H. MARKS, S. DUTTA, J. GAUTHIER and D. ELLIOTT, *J. Pharmacol.* **145**, 351 (1964).
30. R. MEGGES and K. REPKE, Lecture read to the Biochem. Symp. Halle, 1962; *Verh. Ges. exp. Med.* **4**, 195 (1963).
31. G. T. OKITA, F. E. KELSEY, E. J. WALAZSEK and E. M. K. GEILING, *J. Pharmacol.* **110**, 244 (1954).
32. G. T. OKITA, P. J. TALSO, J. H. CURRY, JR., F. D. SMITH, JR. and E. M. K. GEILING, *J. Pharmacol.* **113**, 376 (1955).
33. G. T. OKITA, P. J. TALSO, J. H. CURRY, JR., F. D. SMITH, JR. and E. M. K. GEILING, *J. Pharmacol.* **115**, 371 (1955).
34. G. PAFF, *J. Pharmacol.* **69**, 311 (1940).
35. K. PÁVEK and F. V. SELECKÝ, *Bratisl. lék. Listy* **40**, 481 (1960).
36. K. PÁVEK and F. V. SELECKÝ, *Bratisl. lék. Listy* **40**, 551 (1960).
37. M. M. PESEZ, *Annls pharm. fr.* **10**, 104 (1952).
38. K. REPKE, *Naturwissenschaften* **45**, 94 (1958).
39. K. REPKE, *Naturwissenschaften* **45**, 366 (1958).
40. K. REPKE, *Naturwissenschaften* **45**, 20 (1958).
41. K. REPKE, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **233**, 261 (1958).
42. K. REPKE, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **233**, 271 (1958).
43. K. REPKE, in cooperation with S. KLESCZEWSKI and L. ROTH, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **237**, 34 (1959).
44. K. REPKE and F. LAUTERBACH, in cooperation with S. KLESCZEWSKI and L. ROTH, *Verh. dt. Ges. Kreisl Forsch.*t**25**, 290 (1959).
45. K. REPKE, in cooperation with L. ROTH and S. KLESCZEWSKI, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **237**, 155 (1959).
46. A. SJOERDSMA and C. S. FISCHER, *Circulation* **4**, 100 (1951).
47. J. R. SMITH and H. A. FOZZARD, *Nature, Lond.* **197**, 562 (1963).
48. E. H. SONNENBLICK, H. SPOTNITZ and D. SPIRO, *Circulation Res.* **15**, 70 (1964).

49. M. TAKAGI, D. ZANUTTINI, E. KHALIL and S. BELLET, *Am. J. Cardiol.* **15**, 203 (1965), cit. according to *Excerpta med.* Sect. II C **18**, 771 (1965).
50. R. D. TANZ, *Pharmacologist* **2**, 95 (1960).
51. R. D. TANZ, *J. Pharmacol.* **144**, 205 (1964).
52. F. E. TUBBS, L. CREVASSE and M. W. WHEAT, *Circulation Res.* **14**, 236 (1964).
53. P. G. WASER, *Experientia* **18**, 35 (1962).
54. A. WOLLENBERGER and W. HALLE, *Med. ber. dt. Akad. Wiss.* **5**, 38 (1963).
55. S. E. WRIGHT, *The Metabolism of Cardiac Glycosides.* Blackwell, Oxford (1960).