refusal reaction should differentiate the part played by taste and smell in the refusal response.

These experiments show that when rats are exposed to ANTU, which they probably taste only faintly, they are able to protect themselves by a rapid development of both a tolerance and a refusal. It is unlikely that rats ever encounter ANTU or closely allied thiourea compounds in the wild state, but the principle involved in the development of tolerance and refusal probably holds equally true for other poisons.

Summary. 1. Adult domestic Norway rats were found to accept ANTU when mixed with the stock diet in concentrations at least as high as 10%. In all concentrations from 0.1% to 10% inclusive the ANTU-poisoned food killed 100% of the rats. Lower concentrations of ANTU-poisoned food gave a 100% survival.

2. When fed sublethal concentrations of ANTU food from 0.05% to 0.005% inclusive for 14 days, the rats developed a marked tolerance and refusal. Lower concentrations failed to produce either.

3. In some rats the tolerance disappeared at the end of 16 days and was almost imperceptible in all of the rats at the end of 30 days.

4. The refusal decreased after 16 days, though in some animals it was definitely present even at the end of 30 days.

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Further Studies on Relationship Between Activity and Constitution in the Cardiac Glycoside Series.*

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In a recent report[1] various glycosides and genins were compared with respect to their ability to produce atrioventricular block in the isolated embryonic chick heart, and it was possible to suggest certain conclusions as to the relationship between chemical structure and cardiac activity. The technic has been described in detail and a procedure for estimating the precision of the results has been proposed.[2] A number of additional glycosides and related compounds have now been studied and the results are given in Table I. In general, each compound was compared directly with a closely related reference material and indirect comparisons were computed from previously published activity ratios.

For practical reasons it is difficult to study the interrelationships of the glycosides in man. Hence, in comparing these drugs on an artificial preparation it is necessary to inquire into the significance of the results with respect to the observed clinical potencies. Comparison of the oral therapeutic dose for 3 well known drugs with the results obtained by bio-assay showed a direct correlation with the embryonic heart data but divergent results with respect to the cat assay.[1] Thus, the ratios of the human daily maintenance doses for digitoxin, digoxin, and lanatoside C were 1:3:7; the concentration ratios for atrioventricular block in the isolated heart, 1:3:4.5; while for the intravenous lethal doses in the cat the ratios were 1:0.7:0.45. Each of these ratios has been computed on

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1 DeGraff, A. C., Paff, G. H., and Lehman, R. A., *J. Pharmacol.*, 1941, **72**, 211.

2 Lehman, R. A., and Paff, G. H., *J. Pharmacol.*, 1942, **75**, 207.

TABLE I.
Comparison of Glycosides and Derivatives.

Compound	Molecular wt	Reference compound	Potency for embryonic chick heart in terms of reference compound ± standard error (on a molar basis)	Potency by cat assay in terms of reference compound (on a molar basis)*
Convallatoxin	550	Cymarin	1.00 ± 0.12	1.40[3]
Strophanthidin-β-d-glucoside	566	"	0.96 ± 0.037	1.25[3]
Strophanthidin-tetra-acetyl-β-d-glucoside	734	Strophanthidin	1.20 ± 0.098	0.31[4]
Strophanthidin-3-acetate	462	"	1.20 ± 0.074	1.99[3]
Strophanthidin-3-n-caproate	518	"	1.45 ± 0.15	1.94[3]
Periplocymarin	534	Cymarin	1.09 ± 0.12	0.70[3]
Thevetin	867	Digitoxin	0.040 ± 0.0030	0.42[3]
Ouabain	584	"	0.121 ± 0.009 0.144 ± 0.012	2.14[3]
Gitalin (amorphous)	668	"	0.550 ± 0.040 0.575 ± 0.036	0.38[5]
α-β-angelica lactone	98	"	0.0021 ± 0.00012	—[6]
β-γ-angelica lactone	98	"	0.0013 ± 0.00011	—[6]
Cerberin	522	"	1.64 ± 0.17	1.51[3]

* Values in this column have beeen computed from the lethal dose in the literature for the individual glycosides and do not represent direct comparisons as do the embryonic chick heart data.

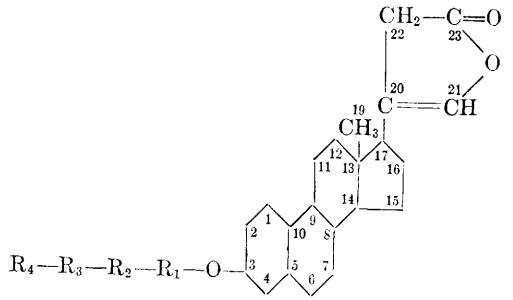
TABLE II.
Summary of Factors Which Modify the Inherent Potency of the Glycosides as Estimated by Various Methods.

	Latent period	Absorption from the gastrointestinal tract	Ability of the heart to remove the glycoside from the blood stream in competition with dissipation processes
Oral maintenance dose in man	Little or no significance	Importance questionable; there has been no direct estimation of the rate and completeness of absorption.	Probably the determining factor.
Lethal dose by intravenous infusion in the cat within 60 to 90 min.	Very important; the longer the latent period the less potent the drug appears.	Not involved.	Relatively unimportant because of the rapid introduction of the drug into the blood stream and the high concentration to which the heart is subjected.
A-V block concentration in the isolated embryonic chick heart	No significance.	" "	Removal of drug from the surrounding medium probably very important; dissipation is not involved.

a molar basis but the discrepancy would be the same in terms of weight. The explanation for this is probably to be found in the relative importance of the factors which modify the inherent potency of these drugs when estimated by different procedures. These factors are summarized in Table II which serves to emphasize the incompleteness of our knowledge and suggests that the isolated heart method may at least measure one of the more important factors which determine the oral dose in man. On the other hand, it is evident that the cat assay gives major emphasis to a factor which is

of no significance in man by this route of administration. If a table of cat unit potencies be examined, such as that in a recent review by Chen,[3] the shortcomings of this method of evaluation become immediately apparent. Thus, digitoxin appears to have the same potency as strophanthidin (0.325 ± 0.011 and 0.325 ± 0.023 mg per kg) and to be only slightly more potent than its own genin (0.459 ± 0.036). By the chick heart method digitoxin was found to be 9.6 times strophanthidin and 8.9 times digitoxigenin.[1] Similar comparisons are given below.

Results. The basic skeleton of the glycosides is



The following observations are based on the data of Table I:

The Substituent on C-3. In the naturally occurring glycosides a carbohydrate residue is attached to the ring through the OH group on C-3. This residue may be a single desoxyhexose or made up of 2, 3 or 4 sugar units. It is generally recognized that such a substituent is essential to secure the "cumulative" property characteristic of these drugs. "Cumulative" is here used in the sense of the earlier work on the relative ease of washing the glycosides out of the heart. As suggested in Table II, the extent to which this property is taken into account in the estimation of potency depends upon the method used. The embryonic heart method appears to give considerable weight to this factor as indicated by a 9- to 37-fold increase in potency when various glycosides were compared with their genins. However, Chen has concluded that, "Different sugars may intensify the cardiac action to a different degree."[3] This does not appear to follow in the isolated heart so long as only a single sugar unit is involved. Strophanthidin cymaroside (Cymarin), strophanthidin rhamnoside (Convallatoxin) and strophanthidin-β-d-glucoside (synthetic) are identical in activity within the limits of experimental error. As more sugar units are added to the molecule, however, the potency decreases somewhat. Complete blocking of the OH groups of the sugar residue such as by acetylation of strophanthidin-β-d-glucoside to give the tetra acetyl derivative, yields a compound with little greater activity than the parent genin.

Attempts have been recently made to substitute other groups at C-3. A theophylline derivative of scillaridin was found to be active but the compound was never adequately characterized.[4,5] Many synthetic esters of strophanthidin have been studied, especially by Chen[4,9] and Gold[10] and their coworkers. Results are included in Table I for the acetate and caproate which were found to be slightly more potent than strophanthidin. This agrees with Gold's findings that their action in man by oral administration is very weak, which he considers to be due to poor absorption but concedes, "There is the possibility that more might have been absorbed but the very rapid elimination kept pace with a relatively slow absorption."

Other Structural Relationships. (1) Periplocymarin and cymarin, which differ from

[3] Chen, K. K., *Annual Review of Physiology*, Annual Reviews, Inc., Stanford University, 1945, Vol. VII, p. 677.

[4] Chen, K. K., and Elderfield, R. C., *J. Pharmacol.*, 1942, **76**, 81.

[5] *New and Non-Official Remedies*, American Medical Association, 1946, p. 329.

[6] Chen, K. K., Steldt, F. A., Fried, J., and Elderfield, R. C., *J. Pharmacol.*, 1942, **74**, 381.

[7] Riseman, J. E. F., and London, S. B., *Science*, 1940, **92**, 384.

[8] DeGraff, A. C., and Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 36.

[9] Steldt, F. A., Anderson, R. C., Maze, N., and Chen, K. K., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 198.

[10] Gold, H., Otto, H. L., Modell, W., and Halpern, S. L., *J. Pharmacol.*, 1946, **86**, 301.

each other only in having respectively a —CH_3 and a —CHO group on C-10, are of identical potency. This is in fair agreement with cat assay results if allowance is made for experimental error.

(2) A daily oral dose in man of 1.3 to 3.7 mg has been suggested for thevetin[11] as compared with 0.1 to 0.2 mg for digitoxin[12] or an average molar ratio of about 1/15. In the isolated heart thevetin is 1/25 as potent as digitoxin, which is in much better agreement than is the cat assay for which the ratio is about 1/2. The genins corresponding to these 2 drugs are stereo-isomeric and different triosides are attached at C-3. The influence of the stereochemical arrangement may be judged if it is assumed that this difference in the sugars is of minor significance.

(3) Ouabain is one-eighth as active as digitoxin. In man, ouabain is considered an unreliable drug by the oral route but it has never been determined with certainty whether this is due to poor absorption, rapid dissipation or failure to "cumulate." Ouabain is twice as potent as digitoxin in the cat.

(4) Amphorous gitalin from the water-soluble fraction of Digitalis purpurea has about half the potency of digitoxin by either method. Stroud and coworkers[13] found the daily oral dose of gitalin to be approximately 0.27 mg, which gives a molar ratio to digitoxin of about 0.49.

(5) The 2 angelica lactones show an activity 1/800 to 1/500 that of digitoxin. Furthermore, their effect on the heart is qualitatively different from the glycosides in that ventricular arrest frequently appears before and instead of atrioventricular block. This contributes further evidence that the lactone ring is only part of the structural configuration necessary for the typical effects of the cardiac glycosides. These compounds are not considered to have digitalis-like action in the cat.

(6) Cerberin is a crystalline glycoside of uncertain structure, which, like gitalin, has about the same ratio to digitoxin by either method.

Summary. The potency of a number of glycosides and related compounds for the embryonic chick heart has been reported and various implications suggested. As a means of studying the relationship of chemical constitution and physiologic action, the embryonic heart method appears to offer definite advantages over methods employing intravenous administration to intact animals.

11 Middleton, W. S., and Chen, K. K., *Am. Heart J.*, 1936, **11**, 75.

12 Gold, H., Kwit, N., and Cattell, McK., *J. Pharmacol.*, 1940, **69**, 177.

13 Stroud, W. D., Livingston, A. E., Bromer, A. W., Vander Veer, J. B., and Griffith, G. C., *Ann. Int. Med.*, 1934, **8**, 710.

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Failure of Tyrothricin in the Treatment of Experimental Syphilis of Rabbits.

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From the standpoint of antibiotic therapy the possible therapeutic effectiveness of tyrothricin in the treatment of experimental syphilis of rabbits is not without interest. Owing to its toxicity by intravenous injection, however, largely due to hemolytic activity, the compound has not been generally employed in the treatment of experimental infections of the lower animals by this route of administration.

In these experiments a 2% alcoholic solution of tyrothricin (Parke-Davis Co.) was employed, suspensions being freshly prepared by diluting 1 cc with 99 cc of sterile distilled