

in all likelihood of gitoxigenin) renders the latter practically non-toxic. A few similar comparative toxicity tests which we have made with tetrahydroouabain have shown it to be at least one hundred times less toxic for frogs than ouabain.

From these observations there is a very strong suggestion that the aglucones of ouabain, the digitalis glucosides and perhaps of other substances of this pharmacological group such as bufotalin, possess, like strophanthidin, an unsaturated lactone group; and that this group may be essential, perhaps in conjunction with other structural features, for the pharmacodynamic action of these substances. We are at present attempting to ascertain by more direct chemical methods, as was accomplished in the case of strophanthidin, whether these substances are indeed inner esters of enolized ketones; and we are also attempting to substantiate by further work the suggested pharmacodynamic significance of the unsaturated lactone group.

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**Availability of synthetic media for streptococci.**

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Six hundred and seventy-one synthetic media were tested to determine their availability for streptococci. They may be grouped into three series: (1) those testing the availability of carbon compounds (carbohydrates and related substances, glycerol, and organic acids such as lactic, malic, tartaric and citric); (2) those testing the availability of nitrogen compounds (the common amino acids, caffeine, betaine, urea, and inorganic ammonium salts such as  $(\text{NH}_4)_2\text{CO}_3$ ,  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{HPO}_4$ ); (3) those testing the availability of some inorganic substances (compounds of Ca, Na, K, and Fe, and S). Most of the media were those previously used for other organisms by different in-

investigators. The composition of the media ranged from water solutions of single substances to combinations of ten different materials.

Estimate of Growth	1st Transplant Subcultures						2nd Transplant Subcultures					3rd Transplant Subculture				4th Trans. Subcultures			5th Tr. Subcult.		6th Tr. Subcult.
	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	1	2	3	1	2	1
4+	103	66	29	19	11	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3+	192	103	80	56	33	23	0	4	2	1	0	0	0	0	0	0	0	0	0	0	0
2+	85	98	80	61	52	29	6	6	2	1	1	0	0	0	0	0	0	0	0	0	0
1+	52	45	42	36	41	31	81	34	27	25	21	0	0	1	1	0	0	0	0	0	0
Several Colonies	9	16	13	5	8	4	72	44	31	11	8	1	1	0	0	0	0	0	0	0	0
Total No. of media showing growth	441	328	244	177	145	93	159	88	62	38	30	1	1	1	1	1	0	0	0	0	0
Percent of media showing growth	66.5	49.7	39.3	28.6	22.0	14.1	25.6	13.3	9.4	5.7	4.5	0.1	0.1	0.1	0.1	0	0	0	0	0	0

Four hundred and forty-one permitted the streptococci to remain viable through one or more transplants. While it would seem from this, perhaps, that it is a comparatively simple matter to manufacture synthetic mixtures which will permit the growth of these organisms, our results indicate a marked deficiency in all of these media. The significant details may be summarized in the following table in which the figures recorded indicate the number of media showing living organisms in the various subcultures, as explained below:

The symbols, 4+, 3+, 2+, 1+, and the phrase "several colonies," are used to indicate the amount of growth; 4+ denotes the maximum. The tests on each medium included six transplants. The one from the "test suspension" (consisting of streptococcus growth removed from infusion agar with a spatula and suspended in 0.9 per cent NaCl solution) to the synthetic medium is designated as the first transplant; the five others are successive transplants at 24 hour intervals from synthetic medium to synthetic medium. The subcultures are transfers from synthetic medium to infusion agar in order to ascertain whether there were live organisms in the synthetic medium. Each successive subculture was made 24 hours after the one immediately preceding. Thus subculture 1 was made after 24 hours incubation of the transplant under consideration; subculture 2 after 48 hours, subculture 3 after 72 hours, etc.

The data in the table indicate: (1) a decrease in the per cent of media showing living organisms in successive transplants; (2) a very marked decrease in the number of "positives" in the successive subcultures of the first transplant series; (3) a change that is not so abrupt in the second transplant series; (4) only one medium that shows growth in the third transplant series; and (5) no medium showing growth in the fourth, fifth, or sixth transplants.

The media do not permit the physiological functions of this organism to continue normally; hence propagation decreases and finally ceases. Continued growth in the first two transplants may be due either to a small reserve store of necessary nutrient (nutrients?) or to the transfer of small amounts of some of the constituents of the conventional medium (infusion agar) on which the test culture was amassed.