Section on Scientific Papers

Papers Presented at the Sixtieth Annual Convention

VARIATION IN THE SUSCEPTIBILITY OF THE GUINEA PIG TO THE HEART TONIC GROUP.

(Second Paper.)

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In a paper read at the 1911 meeting of the American Pharmaceutical Association in Boston, one of us outlined a plan for a series of experiments to cover a period of one year, during which time the susceptibility to Ouabain poisoning of guinea pigs, male and female, large and small, might be observed. During the year which has elapsed since that meeting, this set of experiments has been carried out, following the plan outlined in the former paper just as closely as it was possible to do so.

As stated in the first paper, the experimental pigs were divided into four classes, males large and small, and females large and small. All were fed alike on oats, hay and seasonable greens, as previous experience had demonstrated that pigs cannot thrive on greens alone. All were housed alike, being brought in from their country quarters in groups of fifty to one hundred, and kept in specially provided quarters in the laboratory for one to two weeks before using for the tests.

The test substance used was Merck's Crystallized Strophanthin, Thoms (Ouabain), a fresh solution from the same sample being prepared for each series of injections, in order to obviate as far as possible any deterioration of the material.

The following tables show in detail the results of the tests month by month. The doses are in all cases those given per 250 gram weight:

JULY, 1911.

Temperature of Laboratory 25 to 29° C. Aver. 27°C.

Small Males, 140 to 210 gms.

Dose		Results
0.0000400	—	Recovered
0.0000440	—	Recovered
0.0000470	—	Recovered
0.0000500	—	Recovered
0.0000500	—	Recovered
x0.0000525	+	Died
0.0000550	+	Died
0.0000575	+	Died
0.0000600	+	Died
0.0000690	+	Died
0.0000720	+	Died

M. L. D.=0.0000525.

Large Males, 270 to 410 gms.

Dose		Results
0.0000375	—	Recovered
0.0000400	—	Recovered
0.0000440	—	Recovered
0.0000470	—	Recovered
0.0000470	+	Died*
0.0000500	—	Recovered
0.0000500	—	Recovered
x0.0000525	+	Died
0.0000550	+	Died
0.0000600	+	Died

M. L. D.=0.0000525.

*Died "out of order."

Small Females, 160 to 210 gms.

Dose		Results
0.0000400	—	Recovered
0.0000440	—	Recovered
0.0000440	—	Recovered
0.0000470	—	Recovered
0.0000470	—	Recovered
0.0000500	—	Recovered
0.0000500	+	Died
x0.0000525	+	Died
0.0000550	+	Died
0.0000575	+	Died
0.0000600	+	Died

M. L. D.=0.0000525.

Large Females, 260 to 350 gms.

Dose		Results
0.0000375	—	Recovered
0.0000400	—	Recovered
0.0000400	—	Recovered
0.0000440	—	Recovered
0.0000440	—	Recovered
0.0000470	—	Recovered
0.0000470	—	Recovered
x0.0000500	+	Died
0.0000500	+	Died
0.0000525	+	Died
0.0000600	+	Died

M. L. D.=0.0000500.

AUGUST, 1911.

Temperature of Laboratory 25 to 29° C. Aver. 27° C.

Small Males, 140 to 210 gms.

Dose		Results
0.000040	—	Recovered
0.000044	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
x0.0000525	+	Died
0.000055	+	Died
0.0000575	+	Died

M. L. D.=0.0000525.

Large Males, 270 to 410 gms.

Dose		Results
0.000040	—	Recovered
0.000044	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
x0.0000525	+	Died
0.000055	+	Died

M. L. D.=0.0000525.

Small Females, 160 to 210 gms.

Dose		Results
0.000040	—	Recovered
0.000044	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
x0.0000525	+	Died
0.0000550	+	Died
0.0000575	+	Died

M. L. D.=0.0000525.

Large Females, 260 to 345 gms.

Dose		Results
0.000040	—	Recovered
0.000044	—	Recovered
0.000047	—	Recovered
x0.000050	+	Died
0.000050	+	Died
0.0000525	+	Died

M. L. D.=0.000050.

SEPTEMBER, 1911.

Temperature of Laboratory 20 to 27° C. Aver. 23.5° C.

Small Males, 105-215 gms.

Dose		Results
0.000045	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
0.000050	—	Recovered
0.000050	+	Died
x0.0000525	+	Died
0.0000525	+	Died
0.000054	+	Died
0.000058	+	Died
0.000062	+	Died

M. L. D.=0.0000525.

Large Males, 240 to 320 gms.

Dose		Results
0.000045	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
0.000050	—	Recovered
x0.0000525	+	Died
0.0000525	+	Died
0.000054	+	Died
0.000058	+	Died
0.000062	+	Died

M. L. D.=0.0000525.

Small Females, 105 to 245 gms.

Dose		Results
0.000044	—	Recovered
0.000047	—	Recovered
0.000050	—	Recovered
0.000050	—	Recovered
0.000050	+	Died
x0.0000525	+	Died
0.0000525	+	Died
0.000054	+	Died
0.000058	+	Died

M. L. D.=0.0000525.

Large Females, 230 to 295 gms.

Dose		Results
0.000037	—	Recovered
0.000040	—	Recovered
0.000044	—	Recovered
0.000044	—	Recovered
0.000044	—	Recovered
x0.000047	+	Died
0.000047	+	Died
0.000047	+	Died
0.000050	+	Died
0.0000525	+	Died

M. L. D.=0.000047.

OCTOBER, 1911.

Temperature of Laboratory 18 to 24.5° C. Aver. 21.25° C.

Small Males, 190 to 335 gms.

Dose		Results
0.000047	—	Recovered
0.000050	—	Recovered
0.0000525	—	Recovered
0.000057	—	Recovered
0.000057	—	Recovered
0.000060	—	Very sick—recovered
x0.000060	+	Died
0.000060	+	Died
0.0000625	+	Died

M. L. D.=0.000060.

Large Males, 465 to 745 gms.

Dose		Results
0.000047	—	Recovered
0.000047	—	Recovered
0.000050	—	Very sick—recovered
0.000050	—	Very sick—recovered
0.000050	+	Died*
0.0000525	—	Very sick—recovered
x0.0000525	+	Died
0.0000525	+	Died
0.000055	+	Died
0.0000575	+	Died
0.000060	+	Died

M. L. D.=0.0000525.

*Died "out of order."

Small Females, 225 to 310 gms.

Dose		Results
0.000045	—	Recovered
0.000047	—	Recovered
0.000047	—	Recovered
0.000047	+	Died*
0.000050	—	Recovered
0.0000525.............	—	Very sick—recovered
0.0000525.............	—	Very sick—recovered
0.0000525.............	+	Died
x0.000055	+	Died
0.000057	+	Died
0.000057	+	Died
0.000060	+	Died
0.000060	+	Died

M. L. D.=0.000055.

Large Females, 370 to 705 gms.

Dose		Results
0.0000325.............	—	Recovered
0.0000375.............	—	Recovered
0.000040	—	Recovered
0.000040	—	Recovered
0.000040	—	Very sick—recovered
0.000044	—	Very sick—recovered
0.000044	—	Very sick—recovered
0.000047	—	Very sick—recovered
0.000050	—	Very sick—recovered
x0.000050	+	Died
0.000050	+	Died
0.000052	+	Died
0.000055	+	Died
0.000057	+	Died

M. L. D.=0.000050.

NOVEMBER, 1911.

Temperature of laboratory, 20 to 24 ° C. Aver., 22° C.

Small Males, 175 to 320 gms.

Dose		Results
0.000045	—	Recovered
0.000047	—	Recovered
0.000050	+	Died*
0.000055	—	Recovered
0.000055	—	Recovered
x0.000057	+	Died
0.000057	+	Died
0.000060	+	Died
0.000065	—	Recovered*
0.000070	+	Died
0.0000725.............	+	Died
0.0000725.............	+	Died

M. L. D.=0.000057.

Large Males, 410 to 670 gms.

Dose		Results
0.000050	—	Recovered
0.000055	—	Recovered
0.000057	—	Recovered
0.000057	—	Recovered
x0.000060	+	Died
0.000060	+	Died
0.0000625.............	+	Died
0.000065	+	Died

M. L. D.=0.000060.

Small Females, 165 to 395 gms.

Dose		Results
0.000050	—	Recovered
0.0000525.............	—	Recovered
0.000055	—	Recovered
0.000055	—	Recovered
x0.000057	+	Died
0.000057	+	Died
0.000060	+	Died
0.0000625.............	+	Died
0.000065	+	Died
0.000067	+	Died

M. L. D.=0.00005

Large Females, 380 to 610 gms.

Dose		Results
0.000050	—	Recovered
0.0000525.............	—	Recovered
0.0000525.............	—	Recovered
0.0000525.............	+	Died*
0.000055	—	Recovered
0.000055	—	Recovered
x0.000057	+	Died
0.000057	+	Died
0.000057	+	Died
0.000060	+	Died
0.000065	+	Died

M. L. D.=0.000057.

DECEMBER, 1911.

Temperature of laboratory, 14 to 23° C. Aver., 18.5° C.

Small Males, 160 to 290 gms.

Dose		Results
0.000055	—	Recovered
0.0000575.............	—	Recovered
0.000060	—	Recovered
0.000065	—	Recovered
0.0000675.............	—	Recovered
0.000070	—	Recovered
x0.000070	+	Died
0.000070	+	Died
0.0000725.............	+	Died
0.000075	+	Died
0.000080	+	Died
0.000087	+	Died
0.000090	+	Died
0.000094	+	Died

M. L. D.=0.000070.

Large Males, 350 to 495 gms.

Dose		Results
0.000040	—	Recovered
0.000043	—	Recovered
0.000045	—	Recovered
0.000047	—	Recovered
0.000055	—	Recovered
0.000060	—	Recovered
0.0000625.............	—	Recovered
0.000065	—	Recovered
0.0000675.............	—	Recovered
0.000070	—	Recovered
x0.000070	+	Died
0.000070	+	Died
0.000070	+	Died
0.000075	+	Died
0.000080	+	Died
0.000084	+	Died
0.000087	+	Died
0.000090	+	Died

M. L. D.=0.000070.

* Died or recovered "out of order."

Small Females, 175 to 290 gms.

Dose		Results
0.000055	—	Recovered
0.000060	+	Died*
0.000065	—	Recovered
0.000065	—	Recovered
0.0000675	—	Recovered
0.0000675	—	Recovered
0.0000675	+	Died
x0.000070	+	Died
0.000070	+	Died
0.0000725	+	Died
0.000075	+	Died
0.000080	+	Died
0.000080	+	Died
0.000087	+	Died

M. L. D.=0.000070.

Large Females, 275 to 395 gms.

Dose		Results
0.000060	—	Recovered
0.000065	—	Recovered
0.000065	+	Died*
0.0000675	—	Recovered
x0.000070	+	Died
0.000070	+	Died
0.000075	+	Died
0.000080	+	Died
0.000084	+	Died

M. L. D.=0.000070.

JANUARY, 1912.

Temperature of laboratory, 15 to 25° C. Aver., 20° C.
Temperature of guinea-pig quarters, 7 to 17° C. Aver., 12° C.

Small Males, 170 to 345 gms.

Dose		Results
0.000055	—	Recovered
0.000057	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
0.000060	+	Died*
0.0000625	—	Recovered
x0.0000625	+	Died
0.0000625	+	Died
0.000065	+	Died
0.000065	+	Died
0.0000675	+	Died

M. L. D.=0.0000625.

Large Males, 330 to 790 gms.

Dose		Results
0.000055	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
0.0000625	—	Recovered
x0.0000625	+	Died
0.0000625	+	Died
0.000065	+	Died
0.000065	+	Died
0.000065	—	Recovered*
0.0000675	+	Died
0.000070	+	Died

M. L. D.=0.0000625.

Small Females, 205 to 295 gms.

Dose		Results
0.0000625	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
0.0000675	—	Recovered
0.0000675	—	Recovered
0.0000675	+	Died*
0.000070	—	Recovered
0.000070	—	Recovered
0.000070	+	Died
x0.0000725	+	Died
0.0000725	+	Died
0.0000725	+	Died

M. L. D.=0.0000725.

FEBRUARY, 1912.

Temperature of laboratory, 15 to 22° C. Aver., 18.5° C.
Temperature of guinea-pig quarters, 8 to 16° C. Aver., 12° C.

Small Males, 210 to 360 gms.

Dose		Results
0.000045	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
x0.000065	+	Died
0.000065	+	Died
0.000070	+	Died
0.000070	—	Recovered*
0.000075	+	Died
0.000080	+	Died

M. L. D.=0.000065.

Large Males, 330 to 615 gms.

Dose		Results
0.000065	—	Recovered
0.000070	—	Recovered
0.000070	—	Recovered
0.000070	+	Died*
0.000072	—	Recovered
0.000075	—	Recovered
0.000075	—	Recovered
x0.000075	+	Died
0.000080	+	Died
0.000080	+	Died
0.000085	+	Died
0.000090	+	Died
0.000092	+	Died
0.000095	+	Died

M. L. D.=0.000075.

* Died or recovered "out of order."

Small Females, 175 to 320 gms.

Dose		Results
0.000060	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
0.000070	—	Recovered
0.000070	—	Recovered
0.000070	+	Died*
0.000075	—	Recovered
0.000075	—	Recovered
0.000075	+	Died*
0.000080	—	Recovered
0.000080	—	Recovered
0.0000825	—	Recovered
0.0000825	—	Recovered
0.0000825	+	Died*
0.000085	—	Recovered
0.000085	—	Recovered
x0.000085	+	Died
0.000085	+	Died
0.000085	+	Died
0.0000875	+	Died
0.0000875	+	Died
0.0000875	—	Recovered*
0.000090	+	Died
0.000090	+	Died
0.000090	—	Recovered*
0.000095	+	Died
0.000095	+	Died
0.000100	+	Died

M. L. D.=0.000085.

Large Females, 280 to 410 gms.

Dose		Results
0.000060	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
0.000065	+	Died
x0.000070	+	Died
0.000070	+	Died
0.000075	+	Died
0.000080	—	Recovered*
0.0000825	+	Died
0.000085	+	Died
0.000090	+	Died
0.000095	+	Died

M. L. D.=0.000070.

MARCH, 1912.

Temperature of laboratory, 18 to 22° C. Aver., 20° C.
Temperature of guinea-pig quarters, 10 to 15° C. Aver., 12.5° C.

Small Males, 200 to 390 gms.

Dose		Results
0.000040	—	Recovered
0.000050	—	Recovered
0.000060	—	Recovered
0.000080	—	Recovered
0.0000825	—	Recovered
0.0000825	—	Recovered
0.000085	—	Recovered
0.000085	—	Recovered
0.000085	—	Recovered
0.0000875	—	Recovered
0.0000875	—	Recovered
0.0000875	+	Died
x0.000090	+	Died
0.000090	+	Died
0.000090	—	Recovered*
0.000095	+	Died

M. L. D.=0.000090.

Large Males, 310 to 810 gms.

Dose		Results
0.000060	—	Recovered
0.000065	—	Recovered
0.0000675	—	Recovered
0.000070	—	Recovered
0.0000725	—	Recovered
0.0000725	+	Died*
0.0000725	+	Died*
0.0000725	—	Recovered
0.000075	—	Recovered
0.000075	—	Recovered
0.000075	—	Recovered
0.000075	+	Died*
0.0000775	—	Recovered
0.0000775	—	Recovered
0.0000775	+	Died
x0.000080	+	Died
0.000080	+	Died
0.0000825	+	Died
0.000085	+	Died
0.0000875	+	Died

M. L. D.=0.000080.

Small Females, 215 to 355 gms.

Dose		Results
0.000060	—	Recovered
0.000070	—	Recovered
0.000080	—	Recovered
0.0000825	—	Recovered
0.0000825	—	Recovered
0.0000825	—	Recovered
x0.000085	+	Died
0.000085	+	Died
0.000085	+	Died
0.0000875	+	Died
0.0000875	—	Recovered*
0.000090	+	Died

M. L. D.=0.000085.

Large Females, 265 to 485 gms.

Dose		Results
0.000055	—	Recovered
0.000060	—	Recovered
0.000065	+	Died*
0.0000675	+	Died*
0.0000675	—	Recovered
0.000070	—	Recovered
0.000070	+	Died*
0.0000725	—	Recovered
x0.000075	+	Died
0.000075	+	Died
0.000080	+	Died
0.0000825	+	Died
0.000085	+	Died
0.0000875	+	Died

M. L. D.=0.000075.

*Died or recovered "out of order."

APRIL, 1912.

Temperature of laboratory, 20 to 28° C. Aver., 24° C.
Temperature of guinea-pig quarters, 14 to 20° C. Aver., 17° C.

Small Males, 185 to 250 gms.

Dose		Results
0.000070	—	Recovered
0.000075	—	Recovered
0.0000775.............	—	Recovered
x0.000080	+	Died
0.000080	+	Died
0.0000825.............	+	Died
0.000085	+	Died

M. L. D.=0.000080.

Large Males, 240 to 545 gms.

Dose		Results
0.000065	—	Recovered
0.000070	—	Recovered
0.0000725.............	—	Recovered
0.0000725.............	—	Recovered
0.000075	—	Recovered
0.000075	+	Died*
0.0000775.............	—	Recovered
0.0000775.............	—	Recovered
0.0000775.............	—	Recovered
0.000080	—	Recovered
0.000080	—	Recovered
0.000080	+	Died*
0.0000825.............	—	Recovered
x0.0000825.............	+	Died
0.0000825.............	+	Died
0.000085	+	Died
0.000085	+	Died

M. L. D.=0.0000825.

Small Females, 180 to 310 gms.

Dose		Results	Dose		Results
0.000070	—	Recovered	0.0000775.............	+	Died
0.0000725.............	—	Recovered	0.000080	+	Died
0.0000725.............	+	Died*	0.000080	+	Died
0.000075	—	Recovered	0.0000825.............	+	Died
0.000075	—	Recovered	0.000090	+	Died
x0.0000775.............	+	Died			

M. L. D.=0.0000775.

MAY-JUNE, 1912.

Temperature of laboratory, 23 to 28.5° C. Aver., 25.75° C.
Temperature of guinea-pig quarters, 22 to 29° C. Aver., 25.5° C.

Small Males, 195 to 305 gms.

Dose		Results
0.000065	+	Died*
0.000070	—	Recovered
0.000075	—	Recovered
0.000080	—	Recovered
0.000085	—	Recovered
0.000090	—	Recovered
0.000095	—	Recovered
x0.000095	+	Died
0.000095	+	Died
0.000100	+	Died
0.000100	+	Died
0.000100	+	Died

M. L. D.=0.000095 .

Large Males, 265 to 375 gms.

Dose		Results
0.000065	—	Recovered
0.000065	—	Recovered
0.000067	—	Recovered
0.000070	—	Recovered
0.000070	—	Died*
0.0000725.............	—	Recovered
0.000075	—	Recovered
0.000075	+	Died*
0.000080	—	Recovered
x0.0000825.............	+	Died
0.0000825.............	+	Died
0.000085	+	Died
0.000090		Died

M. L. D.=0.0000825.

Small Females, 210 to 350 gms.

Dose		Results
0.000060	—	Recovered
0.000060	—	Recovered
0.0000625.............	—	Recovered
0.0000625.............	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
0.000070	—	Recovered
0.000070	—	Recovered
0.000070	—	Died*
0.0000725.............	—	Recovered
0.000075	—	Recovered
x0.000075	+	Died
0.000075	+	Died
0.000080	+	Died

M. L. D.=0.000075.

Large Females, 265 to 375 gms.

Dose		Results
0.000065	—	Recovered
0.000067	—	Recovered
0.000070	—	Recovered
0.0000725.............	—	Recovered
0.000075	—	Recovered
0.000080	—	Recovered
0.000085	—	Recovered
x0.000085	+	Died
0.000085	+	Died
0.0000875.............	+	Died
0.000090	+	Died

M. L. D.=0.000085.

* Died "out of order."

JULY, 1912.

Temperature of laboratory, 26 to 30° C. Aver., 28° C.
Temperature of guinea-pig quarters, 22 to 29° C. Aver., 25.5° C.

Small Males, 175 to 375 gms.

Dose		Results
0.000040	—	Recovered
0.000050	—	Recovered
0.000060	—	Recovered
x0.000065	+	Died
0.000065	+	Died
0.000070	+	Died
0.000070	+	Died
0.000075	—	Recovered*
0.000080	+	Died
0.000085	+	Died
0.000090	+	Died
0.0000925	+	Died
0.000095	+	Died
0.000100	+	Died

M. L. D.=0.000065.

Large Males, 335 to 535 gms.

Dose		Results
0.000050	—	Recovered
0.000050	—	Recovered
0.000060	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
x0.000070	+	Died
0.000070	+	Died
0.000080	+	Died
0.000085	+	Died
0.000090	+	Died
0.0000925	+	Died
0.000095	+	Died
0.000100	+	Died

M. L. D.=0.000070.

Small Females, 175 to 360 gms.

Dose		Results
0.000040	—	Recovered
0.000050	—	Recovered
0.000060	—	Recovered
0.0000625	—	Recovered
0.0000650	+	Died*
0.000070	—	Recovered
x0.000075	+	Died
0.000075	+	Died
0.000075	+	Died
0.000080	—	Recovered*
0.000080	—	Recovered*
0.000085	+	Died
0.000090	+	Died
0.000090	—	Recovered*
0.0000925	—	Recovered*
0.0000925	+	Died
0.000095	+	Died
0.000095	+	Died
0.000100	+	Died

M. L. D.=0.000075.

Large Females, 275 to 435 gms.

Dose		Results
0.000040	—	Recovered
0.000045	—	Recovered
0.000050	—	Recovered
0.000060	—	Recovered
0.000060	—	Recovered
0.000065	—	Recovered
0.000065	—	Recovered
0.000065	+	Died
x0.000070	+	Died
0.000070	+	Died
0.000075	+	Died
0.000080	+	Died
0.000090	+	Died
0.000090	—	Recovered*
0.0000925	+	Died
0.000095	+	Died

M. L. D.=0.000070.

* Died or recovered "out of order."

In order to draw conclusions from the above tables it is desirable to condense the results into a single table as follows:

MINIMUM LETHAL DOSES BY MONTHS.

	Small Males	Large Males	Small Females	Large Females	Average
July, 1911. Lab. 27° C.	0.0000525	0.0000525	0.0000525	0.0000500	0.0000519
Aug., 1911. Lab. 27° C.	0.0000525	0.0000525	0.0000525	0.0000500	0.0000519
Sept., 1911. Lab. 23.5° C.	0.0000525	0.0000525	0.0000525	0.0000470	0.0000511
Oct., 1911. Lab. 21.25° C.	0.0000600	0.0000525	0.0000550	0.0000500	0.0000544
Nov., 1911. Lab. 22° C.	0.0000570	0.0000600	0.0000570	0.0000570	0.0000577
Dec., 1911. Lab. 18.5° C.	0.0000700	0.0000700	0.0000700	0.0000700	0.0000700
Jan., 1912. Lab. 20° C. Pens 12° C.	0.0000625	0.0000625	0.0000725	....	0.0000658
Feb., 1912. Lab. 18.5° C. Pens 12° C.	0.0000650	0.0000750	0.0000850	0.0000700	0.0000737
March, 1912. Lab. 20° C. Pens 12.5° C.	0.0000900	0.0000800	0.0000850	0.0000750	0.0000825
April, 1912. Lab. 24° C. Pens 17° C.	0.0000800	0.0000825	0.0000775	....	0.0000800
May-June, 1912. Lab. 25.75° C. Pens 25.5° C.	0.0000950	0.0000825	0.0000750	0.0000850	0.0000844
July, 1912. Lab. 28° C. Pens 25.5° C.	0.0000650	0.0000700	0.0000750	0.0000700	0.0000700
Average	0.0000668	0.0000660	0.0000678	0.0000624	0.0000661

A study of the above table discloses many interesting facts. The results taken month by month naturally fall into four periods. There was scarcely any variation during the months of July to November, inclusive, the average M. L. D. for all classes of pigs running 0.0000519, 0.0000519, 0.0000511, 0.0000544, and 0.0000577. For December and January, these figures increase rather suddenly to 0.0000700 and 0.0000658, respectively. For February, March, April and May-June, they still further increase to 0.0000737, 0.0000825, 0.0000800, and 0.0000844, the high mark. For July, they drop again to 0.0000700.

That this variation might have been caused by any change in the test substance is hardly conceivable. As already stated, the ouabain crystals were kept in dry form—fresh solutions being prepared for each series of tests. To guard against possible mistakes in making up these solutions, the results were in several instances checked against new solutions. Moreover, it is not at all probable that ouabain in dry crystalline form, would remain unchanged for a period of five summer and fall months and then deteriorate during the cold winter months. To make sure of this, however, a new sample of ouabain was procured in July, 1912, and tests were run on this in comparison with the old sample, whereby practically identical results were obtained. Moreover, the melting points of both samples were determined and found to be the same, namely 188 to 189.5° C.

The cause or causes of the variation seem therefore to be narrowed down to the questions of temperature and season, for the average M. L. D. for each of the classes of pigs, during the whole year are nearly identical, namely:

Small males	0.0000668
Large males	0.0000660
Small females	0.0000678
Large females	0.0000624

That for the large females is slightly lower owing to the fact that no pigs of this class were available for the tests during two of the months when the results on the other classes were high. It is apparent, therefore, that sex and size may be disregarded and that just as nearly uniform results will be obtained, if indeed, not more so, by running a sufficient number of pigs in each test, eliminating the factor of sex, and basing all doses on 250 gm. of animal weight.

But not so with the questions of temperature and season. These factors undoubtedly play an important part in the cause of the observed variation but the data so far obtained do not in any satisfactory way make clear just how these factors work. A continuation of this study will be made in order to throw more light on the question.

That the temperature alone is the cause of the variation, however, is clearly shown not to be the case. The M. L. D. does not directly respond to observed temperatures, but it may be seen that in a general way, the resistance to ouabain poisoning is greater following a long continued period of cold weather, and is lower following a long period of hot weather. Thus the results of July and August, 1911, were obtained during a protracted spell of the most sultry and oppressive weather noted in Philadelphia in many years. The resistance, however, continued low throughout the following fall months, and it was not until December and January that any noted increase in resistance occurred. Again this resistance having steadily increased under continued cold weather until the

M. L. D. became 0.0000825 in March, this figure was practically maintained throughout the warmer spring months and did not drop again until July, when it went down to 0.0000700.

If sudden changes, or temporarily higher or lower temperatures, alone caused marked changes in resistance, it might be possible to regulate the temperature during the period of a test so as to guarantee uniformity of result. Such, however, is true only to a certain extent as will be shown later. In July, 1911, with an average laboratory temperature during tests of 27° C., the M. L. D. was lower than in July, 1912, with an average laboratory temperature of 28° C. The latter, however, was only temporary and had been preceded by an unusually cool and pleasant spring and cold winter, while the July of 1911 was sultry and humid throughout and was preceded by a hot and oppressive spring. General seasonal conditions therefore play a most important part in this variation in susceptibility.

On the other hand, that marked changes in temperature have an immediate effect on susceptibility is shown by the following experiment. The male pigs, large and small, used in the January, 1912, experiment were found to have a M. L. D. of 0.0000625. This figure was obtained in the usual way by keeping the pigs in their regular city quarters for a week or more where the average temperature during that time was about 12° C. The pigs were then transferred to the laboratory during their 24 hours of testing, where the temperature averaged 20° C. Having a sufficient number of male pigs left over, we determined, therefore, to observe the M. L. D. on this same lot of pigs at the temperature of their quarters, namely 12° C. Accordingly, on the following day, we injected some of these remaining pigs in their quarters and allowed them to remain there during the 24 hours of test. The temperature during this time ranged from 8 to 14° C. and the pigs used varied in weight from 300 to 505 gm. The result was as follows:

Dose		Results	Dose		Results
0.0000400.............	—	Recovered	0.0000500.............	—	Recovered
0.0000450.............	—	Recovered	0.0000550.............	+	Died
x0.0000475.............	+	Died	0.0000600.............	+	Died
0.0000500.............	+	Died	0.0000625.............	+	Died
			0.0000650.............	+	Died

M. L. D.=0.0000475.

As a check on the regular results whereby an M. L. D. of 0.0000625 was obtained, five additional pigs, ranging in weight from 340 to 400 gms. were then transferred to the laboratory, where the temperature ranged from 22 to 24° C. and were then injected and kept during the test, with the same result as was obtained before, as follows:

Dose		Results	Dose		Results
0.0000570.............	—	Recovered	0.0000600.............	—	Recovered
0.0000600.............	—	Recovered	x0.0000625.............	+	Died
			0.0000650.............	+	Died

M. L. D.=0.0000625.

It is apparent therefore that in cold weather the tests should be made in a room at normal temperature—say 22 to 25° C. But on the other hand, pigs inured to colder winter weather, when tested in a comfortably warm place, are more resistant than those which have been subjected for a long time to hot, sultry summer weather.

We are forced to the conclusion that in order to secure the highest possible state of uniformity for the heart stimulants by means of minimum lethal dose guinea pig method, standards should be based upon a standard M. L. D. of ouabain for 250 gm. of animal weight and we would propose for this standard, a dose of 0.000066 gm. crystallized Strophanthin (Thoms), or Ouabain. Each lot of 50 to 100 pigs should be kept for one week under proper conditions of ventilation, feeding, temperature, etc., and the M. L. D. of ouabain, irrespective of size and sex, be determined at normal room temperature. The ratio of this result to the standard M. L. D. of 0.000066 per 250 gm. of animal should then determine the standard for all preparations to be assayed by means of this lot of pigs. Such a procedure would practically eliminate the variation of 22.7% below to 27.7% above, which represents the extreme range of variation observed during the whole year's experiments. That standarization without this precaution, however, is a vast improvement over no standarization is apparent when we realize that the natural variation in the heart stimulating drugs amounts to several hundred percent.

We are also forced to the opinion that a complete and satisfactory biologic assay process for the *standardization* of the heart tonics, whether by means of guinea pigs or other mammals, or by means of frogs, is hardly yet feasible, for introduction into the Pharmacopœia. It would be exceedingly difficult to lay down any concise, rule of thumb method of *standardization* which could be followed by one inexperienced or but little experienced in the art, with the hope of getting concordant results, and particularly this is the case when we consider that the Pharmacopœia has the force of inflexible authority stamped upon it by its relationship to State and Federal laws. This does not preclude the possibility, however, of making official certain *minimum* biologic requirements, using guinea pigs or frogs, to guard against fraudulent, inert or badly deteriorated drugs and preparations.

A further consideration of the cost of the guinea-pig method is appropriate at this time. The advocates of the frog method for the standardization of the digitalis series criticise the Reed and Vanderkleed guinea pig method on the ground that it is too costly as compared with the frog method. Haskell[1] says: "There can be no question as to the economy of the different methods. Frogs for an assay cost us fifty cents, guinea pigs would cost us about $4.00." Worth Hale[2] says: "Frogs for an assay would cost approximately 50 cents. If mammals are used on the basis that at least three should be used for each assay, the cost would be from $2.00 to $3.00." Walters & Haskell[3] say: "In the assay of the digitalis preparations by the 'one hour' frog method we practically never use more than 18 frogs, the cost of which varies from 40 to 75 cents. Our ignorance of the routine use of the guinea pig method prevents us from stating definitely the cost of an assay. It scarcely seems probable, however, that on an average, less than six or eight animals would suffice. The guinea pigs we have been able to obtain have cost us from 40 to 75 cents apiece, making the probable cost of an assay from $2.00 to $6.00, about 800 percent greater than the frog method."

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[1] Physiologic Drug Testing. J. A. Ph. A., July, 1912.
[2] Hygienic Lab. Bulletin No. 74, p. 15.
[3] Susceptibility of the guinea pig to poisoning by digitalis. J. A. Ph. A., July, 1912.

It is perfectly apparent that these writers all calculate the cost of one assay by multiplying the *number of animals used* by the value of one animal. In our routine work, however, we use the animals over and over until they are killed, only requiring that four or five days elapse between injections and that they appear normal. The fact that it is possible not only to use the pigs over and over but also to obtain accurate results thereby, has been proven by us many times, by determining the M. L. D. of the same preparation both on "used" and "unused" pigs. We have further proven this by using "unused" and "used" pigs in the same series. In all cases, the results obtained from both classes of pigs have been practically concordant.

Following are typical examples of the results obtained from these experiments:

Experiment No. 1: The doses given represent grams of ouabain per 250 gm. body weight of animal:

USED PIGS.
Temperature in laboratory 15 to 25° C.
Pigs varied in weight from 205 to 295 Gms.

Dose	Results	Dose	Results
0.0000625	Recovered	0.0000675	Recovered
0.000065	Recovered	0.000070	Recovered
0.000065	Recovered	0.000070	Recovered
0.0000675	Died *	x0.0000725	Died
0.0000675	Recovered	0.0000725	Died
		0.0000725	Died

M. L. D.=0.0000725.

* Died "out of order."

UNUSED PIGS.
Temperature in laboratory 15 to 22° C.
Pigs varied in weight from 235 to 335 Gms.

Dose	Results	Dose	Results
0.00004	Recovered	0.0000625	Died *
0.00005	Recovered	0.000065	Recovered
0.000055	Recovered	0.000065	Recovered
0.00006	Recovered	0.0000675	Recovered
0.00006	Died *	0.0000675	Recovered
0.00006	Recovered	0.00007	Recovered
.0.0000625	Recovered	0.00007	Recovered
.0.0000625	Recovered	x0.0000725	Died
		0.0000725	Died

M. L. D.=0.0000725.

Experiment No. 2:

USED PIGS.
Temperature in laboratory 18 to 24.5° C.
Pigs varied in weight from 210 to 300 Gms.

Dose	Results	Dose	Results
0.000075	Recovered	0.0000725	Recovered
0.000060	Recovered	0.0000725	Recovered
0.0000625	Recovered	0.000075	Recovered
0.000065	Recovered	0.000075	Recovered
0.000070	Recovered	x0.0000775	Died.
0.0000725	Died *	0.000080	Died
		0.000080	Died

M. L. D.=0.0000775.

* Died "out of order."

UNUSED PIGS.
Temperature in laboratory 18 to 24.5° C.
Pigs varied in weight from 210 to 345 Gms.

Dose	Results		
		0.000070	Recovered
0.000057	Recovered	0.0000725	Recovered
0.000060	Recovered	x0.000075	Died
0.0000625	Recovered	0.000075	Died
0.000065	Recovered	0.0000775	Died

M. L. D.=0.000075.

Experiment No. 3: Eight pigs which had previously been used for testing antitoxin were divided into two lots of four pigs each. These were injected with sub-minimum lethal doses of 0.00003 gm. and 0.00004 gm., respectively, per 250 gm. body weight of animal. The pigs were then mixed, and after allowing three days for recovery, the M. L. D. on these "used" pigs was determined in comparison with a series of "unused" pigs, with the following results:

USED PIGS.
Temperature of laboratory 27 to 30° C.
Pigs varied in weight from 300 to 440 Gms.

Dose	Results	Dose	Results
0.000050	Recovered	0.000075	Recovered
0.000060	Recovered	0.000080	Very sick—Recovered
0.000065	Recovered	x0.0000825	Died
0.000070	Recovered	0.000085	Died
0.0000725	Recovered	0.000090	Died

M. L. D.=0.0000825.

UNUSED PIGS.
Temperature of laboratory 25 to 26° C.
Pigs varied in weight from 255 to 385 Gms.

Dose	Results	Dose	Results
0.000055	Recovered	0.000080	Recovered
0.000060	Recovered	x0.000080	Died
0.000065	Recovered	0.000080	Died
0.000070	Recovered	0.0000825	Died
0.0000725	Recovered	0.000085	Died
0.000075	Recovered	0.000090	Died

M. L. D.=0.00008.

Having proven by the above experiments that it *is* possible to use the pigs repeatedly, *the cost of an assay by the guinea pig method is therefore dependent solely upon the number of pigs killed and not on the number injected.* In order to determine the condition of the heart, with the "one hour" frog method, *all frogs injected must be killed.*

In order to ascertain both the average number of animals used and the average number of animals killed, per assay, by the guinea pig method we went over our laboratory records and found that we had used 3,731 pigs for 333 assays, or an average of 11.2 pigs per assay. Of these 1,742 were killed, making an average of 5.2 pigs per assay. Guinea pigs cost us on an average 40 to 50 cents apiece, making the cost of a single assay range between $2.08 and $2.60. It takes from 14 to 18 frogs for one assay. Frogs cost us on an average of 75 cents to $1.25 per dozen or $1.12 to $1.88 per assay. It can be readily seen, therefore, that the cost of an assay by the guinea pig method is only about 56% greater than by the frog method, instead of 800% greater, as estimated by Walters and Haskell.

Since our experiments have proven that pigs which have first been used for testing antitoxin can be used for assay purposes, it can readily be seen that manufacturers who make antitoxin, can do this work at a still further reduction in cost, as pigs once used in antitoxin standardization cannot be used over again for that purpose.

Attention is also called to the simplicity of determining what the "end reaction" is in the case of the guinea pig M. L. D. method. A review of the detailed tables given in the first part of this paper shows that of the 560 pigs injected in the whole series of tests, only 41, or about 7%, died or recovered "out of order." In no series, except possibly that for small female pigs in July, 1912, would any one have difficulty in concluding what M. L. D. is really indicated. This is not so readily apparent in the case of the "one hour" frog method. In our paper read at Boston last year, we appended some results obtained with frogs from various sources. We have continued these frog experiments but as they are foreign to the scope of this paper, we have presented them in another communication.

SUMMARY.

1. Sex and weight may be dismissed as unimportant in the variation of guinea pigs to ouabain poisoning.

2. Season and temperature are to be regarded as the principal causes of this variation.

3. Guinea pigs inured to cold weather are more resistant to ouabain poisoning than those which have been subjected to a continued season of torrid weather.

4. Increased resistance brought about by acclimation to cold weather persists well into a subsequent heated season, and vice versa.

5. Assays should be conducted at average normal room temperature.

6. To secure the highest degree of accuracy in standardization work, the standard should be based upon a standard minimum lethal dose of 0.000066 gm. ouabain for 250 gm. pigs.

7. The extreme average variation for all classes of pigs ranged from 22.7% below to 27.7% above the average, during the whole year.

8. The cost of the guinea pig assay process depends solely upon the number of pigs killed and not upon the number injected, as pigs which survive may be used over again with accurate results.

9. Out of 560 pigs injected in the series of tests only 7% died out of order, thus rendering easy the determination of the "end reaction."

10. No biologic assay process for the purpose of *standardizing* the heart tonics is advisable for introduction into the Pharmacopœia, but the setting forth of certain *minimum* biologic requirements, to guard against fraudulent, inert, or badly deteriorated drugs, and preparations, is feasible.

PHYSIOLOGIC LABORATORY OF H. K. MULFORD CO., August 6, 1912.

DISCUSSION.

Prof. L. E. Sayre said that this paper was a very valuable presentation of some important subjects to this Association, and he wanted in particular to call attention to the last paragraph, No. 10:

"No biologic assay process for the purpose of *standardizing* the heart tonics is advisable for introduction into the Pharmacopoeia, but the setting forth of certain *minimum* biologic requirements, to guard against fraudulent, inert or badly deteriorated drugs and preparations is feasible."

Prof. Sayre said he wanted to emphasize that statement as meeting his heartiest approval. At one time every one thought diphtheria serum could not be introduced into the Pharmacopoeia with a standard for it. He had championed the idea that it could, and it took that form and was now in the Pharmacopoeia. He hoped that the coming Pharmacopoeia would seriously consider the statement of a biologic method or standard, agreed upon by biologists.

H. C. Hamilton, of Detroit, asked Mr. Vanderkleed if it was not just as necessary to use the standard when guinea-pigs were the test animal as when frogs were the test animal. He said he had not gathered from the presentation of his papers that he had recorded this point.

Mr. Vanderkleed responded that it was to determine that very thing—to throw light on whether or not it was necessary to do that—that this set of experiments had been made. As the report showed, there was a variation of from 22.7 percent below to 27.7 percent above the average, in the whole range. If the guinea-pigs were not standardized, there was a possibility of just so much variation.

Mr. Hamilton said his conclusion from this was, that it was no argument against the use of frogs, necessarily, because the standard would have to be run in both cases.

Mr. Vanderkleed responded that it was so stated in the paper, that this did not preclude the value of the frog method, provided the frogs were standardized. But it did seem to emphasize the greater necessity of standardizing the frog than the guinea-pig. If the guinea-pigs were not standardized, there would probably be a range within 10 or 15 percent, most of the time, whereas with the frogs the variation would be from 200 to 300 or 400 percent, for a difference of time of only one month.

The Chair said that he was unfortunately unable to follow the reading of these papers very closely, and what he had to say might have been discussed in the papers, but he knew that if the frog method was used, it was absolutely necessary to keep the frogs at standard temperature. Then, too, it was necessary to use caution in comparing the variation and the susceptibility of frogs and guinea-pigs. With one poison—for instance, ouabain—as compared with the susceptibility to another poison—for instance, digitalis—the variations did not run the same with frogs and guinea-pigs, and some caution would be necessary. It should not be taken for granted that because frogs and guinea-pigs varied in a certain way with ouabain that they would vary in the same way with digitalis.

WRITING AN ADVERTISEMENT.

To speak to possible customers, you get up a little form, worded as you think to the best advantage, and this is your advertisement. There are certain things that an advertisement should be. Above all, it should be truthful; it should not attempt to mislead or deceive. It should be clear; you are speaking to the lowest intelligence, as well as to the highest. This is not platform oratory, its only object being to get business, and any honest man's money is acceptable to you. An advertisement should be forceful, that is, earnest. In combining these three points, we think you get the basis of a successful advertisement. It should be truthful, clear, and earnest, just exactly the points upon which you would found an argument in talking to a friend.

There are other things that an advertisement may be, in addition to what it should be. It may be humorous, if you wish, and if you are able to tell what humor really is. Smartness is not humor, by a long shot, although people addicted to it evidently think it is. Flippancy is not humor. Humor is a kindly something which enables a man to present an argument in a genial way. The American people are great lovers of humor and things appeal to them which would not appeal to the peoples of other lands. A really humorous advertisement carries weight of its own, and is therefore valuable. A great many advertising experts decry the use of humor, but the writer believes it has its uses and is valuable in its own place.

Humor is an edged tool, however, and must be carefully handled. What you want to be is genial, not humorous at the expense of somebody else. A rough joke nearly always means anything but a joke to its victim. What makes others laugh, makes him squirm. Therefore, do not aim your wit at the religion, or the politics, or the infirmities, of others. These are three great points to be avoided, as you have learned in your daily intercourse with others, and here again we see that talking in an advertisement is much like having a personal conversation with a friend.—*W. S. Adkins in National Druggist.*