TABLE IV.—TOTAL AMOUNT OF INSPECTION CURVE

$n_1 = 50,\ n_2 = 100,\ c_1 = 0,\ c_2 = 2$

$N = 500$

$$TAI(p) = n_1 P_{a_1}(p) + (n_1 + n_2) P_s(p) P_{a_2}(p) + N[1 - P_s(p) P_{a_2}(p) - P_{a_1}(p)]$$

p	$P_{a_1}(p)$	$n_1 P_{a_1}(p)$	$P_s(p) P_{a_2}(p)$	$(n_1 + n_2) \cdot P_s(p) P_{a_2}(p)$	$N[1 - P_s(p) P_{a_2}(p) - P_{a_1}(p)]$	$TAI(p)$
.002	.905	45.25	.093	13.95	1.00	60.20
.005	.779	38.95	.192	28.80	14.50	82.25
.006	.741	37.05	.213	31.95	23.00	92.00
.01	.607	30.35	.251	37.65	71.00	139.00
.02	.368	18.40	.174	26.10	229.00	273.50
.03	.223	11.15	.079	11.85	349.00	372.00
.04	.135	6.75	.030	4.50	417.50	428.75
.048	.091	4.55	.012	1.80	448.50	454.85
.05	.082	4.10	.010	1.50	454.00	459.60
.06	.050	2.50	.003	.45	473.50	476.45
.07	.030	1.50	.001	.15	484.50	486.15
.08	.018	.90	.000	.00	491.00	491.90
.09	.011	.55	.000	.00	494.50	495.05
.10	.007	.35	.000	.00	496.50	496.85

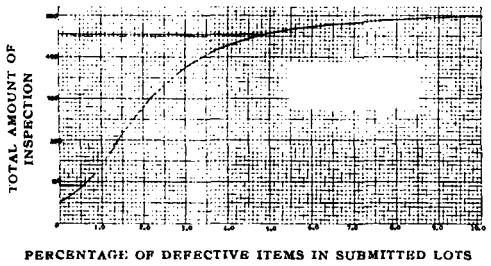

Fig. 5.—Total amount of inspection curve for double sampling inspection plan. $n_1 = 50$, $n = 100$, $c_1 = 0$, $c_2 = 2$. Lot size $N = 500$.

to inspect on the average 18% of every 500 piece lot that comes in at an AQL of .6%, and 91% of every 500 piece lot that comes in at a p_t of 4.8%. (See Table IV and Fig. 5.)

CONCLUSIONS

This paper has attempted to show how a given double sampling plan should be X-rayed to reveal its performance characteristics.

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Notes

Influence of Physostigmine and Neostigmine on the Responses of Goldfish Intestine to Acetylcholine

By THOMAS J. HALEY, G. COLVIN, and M. EFROS

The use of a microbath and electronic amplification of the contractions of goldfish intestine makes it possible to estimate acetylcholine in concentrations of 0.5 to 2.5 $\times 10^{-9}$ Gm. Addition of esterase inhibitors increases the sensitivity of the preparation, but it is not so sensitive as the leech muscle. Physostigmine and neostigmine also cause contractions of the goldfish intestine.

MANY MAMMALIAN intestinal preparations have been used to study the effects of biologically active substances, but Dreyer (1) was the first to describe the effects of acetylcholine and physostigmine on the fish intestine. Euler and Ostlund (2) extended these observations to include other materials, such as histamine, 5-hydroxytryptamine, and substance P. Gaddum and Szerb (3) showed that the intestine of the goldfish (*Carassius auratus*) could be used for such studies. Furthermore, the use of a microbath greatly increased the range of concentrations which could be investigated. However, Gaddum and Szerb (3) did not study the effects of physostigmine or neostigmine on the goldfish intestine. In view of the fact that such compounds generally increase the sensitivity of the intestine

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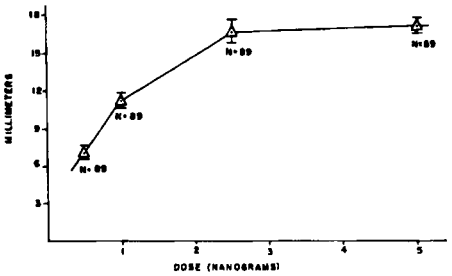

Fig. 1.—Response of the goldfish intestine to acetylcholine in the absence of cholinesterase inhibitors. Mean ± S.E.

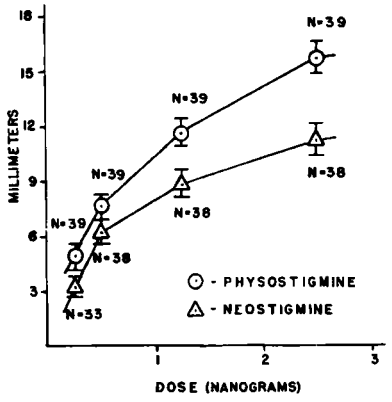

Fig. 2.—Response of the goldfish intestine to acetylcholine in the presence of 250 nanograms of either physostigmine or neostigmine. Mean ± S.E.

TABLE I.—STATISTICAL COMPARISON OF DRUG RESPONSES

Drug	b	S_b^{2a}	t Obtained	t Required at P = 0.05
Acetylcholine, *1*	10.38	1.28	...	...
Acetylcholine + physostigmine, *2*	10.67	2.36	0.152[b]	2.78
Acetylcholine + neostigmine, *3*	7.56	1.74	1.62[c]	2.78

[a] $S_b^2 = \frac{\Sigma[(x - \bar{x})(\text{S.E.})]^2}{[\Sigma(x - \bar{x})^2]^2}$ = estimated variance of slope. [b] Comparison between *1* and *2*. [c] Comparison between *1* and *3*.

to acetylcholine and because we are investigating the release of this substance from brain tissue where low concentrations would be encountered, the decision was made that the effects of both esterase inhibitors should be investigated to determine not only their effects on intestinal contractility but also their influence on intestinal sensitivity to acetylcholine.

EXPERIMENTAL

The microbath and goldfish intestinal preparation described by Gaddum and Szerb (3) was used for the quantitative estimation of acetylcholine. The effects of physostigmine and neostigmine on the acetylcholine response were also investigated. The intestinal contractions were recorded with a Sanborn linear differential transformer model FTA-1-1 and a Sanborn strain gauge amplifier model 140. The ranges of drug concentrations were: acetylcholine 0.25 to 5 nanograms; physostigmine and neostigmine 12 to 500 nanograms. (One nanogram equals 1×10^{-9} Gm.) All drug additions to the microbath were accomplished with a Kensco 50-μl. micropipet. The results were analyzed statistically by calculating the regression lines and comparing the slope values by the t test.

RESULTS

Exposure of the isolated goldfish intestine to acetylcholine resulted in a vigorous contraction with a response that was essentially linear over the dosage range of 0.5 to 2.5 nanograms. Higher doses gave no greater response (Fig. 1). The amount of acetylcholine detected with this preparation was increased when 250 nanograms of either physostigmine or neostigmine were mixed with the acetylcholine (Fig. 2), but the over-all response of the preparation to acetylcholine decreased. The increase in sensitivity was probably related to the inactivation of cholinesterase by the drugs and the decreased over-all response to the direct contractile effects of these drugs on the intestine. Both esterase inhibitors caused slight contractions of the intestine in the absence of exogenous acetylcholine, but the height of contraction was almost identical per dose over the dosage range of 12 to 500 nanograms. Furthermore, there was a tendency for the intestine to remain in a permanent contraction after exposure to doses above 250 nanograms. In all instances conversion to log dose-response curves and comparison of the slope values by the t test indicated no significant difference in the values obtained with or without the esterase inhibitors (see Table I).

DISCUSSION

The results obtained with the goldfish intestine following exposure to acetylcholine confirm those previously reported by others (1–3). The effects of physostigmine and neostigmine were similar to the observations of Dreyer (1). The authors have shown that this preparation gives a linear response over a dosage range of 0.5 to 2.5 $\times 10^{-9}$ Gm. of acetylcholine which is in agreement with Gaddum and Szerb (3). When either physostigmine or neostigmine are added to the solution, the lower limit drops to 0.25 $\times 10^{-9}$ Gm. Using the same technique and the cholinesterase inhibitor isopropylmethylphosphoryl fluoride (sarin), Szerb (4) showed that the leech muscle could detect 25 to 100 $\times 10^{-12}$ Gm. of acetylcholine. Thus, the leech muscle is approximately 10 times more sensitive than the goldfish intestine. However, the availability of the latter to most laboratories may more than compensate for the increased sensitivity of the former. Moreover, the addition of specific antagonists to the bathing fluid makes it possible to eliminate the effects produced by polypeptides, catecholamines, histamine, and other naturally occurring substances (3).

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