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BIONETICS RESEARCH LABORATORIES, INC.
FALLS CHURCH, VIRGINIA

FINAL REPORT

RELATIONSHIP OF EFFECTIVE DOSE TO BODY WEIGHT

For

Army Research Office

3045 Columbia Pike
Arlington, Virginia 22204

Contract No. DA 49-092-ARO-22

Submitted by

BIONETICS RESEARCH LABORATORIES, INC.

Falls Church, Virginia

September 1967

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Introduction

The relationship between the effective dose of a drug and the body weight of the test organism is a subject to which much attention has been devoted. It is, however, extremely difficult to mobilize these discussions because the relevence of a paper is often not revealed in either the title or the summary. Done (1964) in his review, Developmental Pharmacology, has reemphasized the problem. He found relevent papers "included under nearly every conceivable heading in the literature indices." Scanning indices under headings such as dose or body weight is a useless effort. Most citations thus revealed turn out to be reports of the influence of drugs on growth or organ weights. The discussion which follows is almost surely incomplete and an apology is entered at once to those whose work is omitted. Reprints or citations of pertinent papers are solicited.

Certain semantic difficulties should be dealt with first. Much confusion arises from imprecise use of dose versus dosage. In the present paper, the term dose is assigned to the absolute amount of drug administered, while the term dosage is used to indicate an amount adjusted for the individual according to age, weight, or some other factor. It must be remembered that if a relationship exists it may be either direct or inverse, and linear, curvilinear, or nonlinear. It is worth emphasizing that the existence of a mathematical relationship of drug dose to any particular physiologic or anatomic parameter does not justify the implication of biological dependence or a cause and effect relationship.

Two problems arise in efforts to reevaluate previously published information. In the older work, authors usually gave sufficient information about the individuals to allow statistical calculations to be performed, but did not use an adequate number of individuals to justify the calculations. In more recent publications, there is often insufficient detail in the reporting of the original data to allow rearrangement and recalculation by methods other than those chosen by the author.

Many workers have described a relationship between dose and body weight. The character of the observed relationship ranges from exactly direct and linear as reported by Broom, et al (1932), through direct and exponential as detailed by Bliss (1936) among others, to inverse according to Durham, et al (1929). A.J. Clark (1937) cited numerous examples and concluded that different drugs have different relationships, a conclusion well supported by subsequent reports. The relationship of dose to body surface advocated by several authors is aptly characterized by Done (1964) as "a semantic faux pas" since all that is demonstrated is a relationship to a power of body weight, which power happens to be very similar to that which relates surface area to body weight.

Independence of dose from body weight is described for ANTU in rats by Rall and North (1953), for the melanophore expanding action of pituitary extracts in frogs by Deutsch, et al (1956), for histamine in mice by Angelakos (1960), for acetoxycycloheximide in rats by Pallotta, et al (1962), for botulinal and tetanal toxins in mice by Lamanna, et al (1955) (1960), and for dysentery toxin in mice by Zahl, et al (1943).

Experimental investigation of dose to body weight relationships is made difficult by the magnitude of variation from individual to individual. This is so great that many animals within very narrow weight limits must be used to reveal the differences between weight groups as statistically significant. Another problem arises in differentiating differences in weight from differences in age. We do not claim to have solved this problem. We have attempted to cope with it by choosing a range of weight groups which includes both immature and mature animals. Under these circumstances, developmental changes should produce non-linearities in any observed relationship. In spite of the difficulties, it was decided to reexamine a variety of drugs under circumstances which would permit identification of the relationship (if any) of their action to body weight of the test organism.

Method

Drugs to be investigated were chosen to represent one of several pharmacologic characteristics initially felt to be pertinent. These included: previous relevant study, e.g. histamine, ANTU and pentobarbital; action on or selective fixation by some specialized tissue, e.g. tubocurarine, hemicholinium-3, hexamethonium, strychnine, picrotoxin and pentamethylene tetrozol; unusual potency or species specificity, e.g. ANTU, tetrodotoxin and McN-A-343 (an unusual ganglionic stimulant reported upon by Roszkowski [1961]); and conversely, widespread distribution or relatively low potency, e.g. barbital, histamine, 48-80 (histamine liberator), and fluoroacetate. All compounds were purchased from commercial sources with the exception of 48-80 which was supplied by Willcome Research Laboratories, and McN A-343 which was supplied by McNeil Laboratories.

Lethality was chosen as the effect to be studied since relatively simple procedures for the necessary statistical evaluations are widely accepted. Mice were chosen as the test animal so that statistically adequate numbers could be obtained within reasonable economic limitations. The animals were obtained from Dublin Laboratory Animals, Inc., Dublin, Virginia and were members of a strain maintained by random matings within a closed colony. Animals were delivered in groups segregated by sex and within plus or minus one gram of specified weight. Weight groups were specified as 10, 18, and 24 grams for females, and 10, 18, and 26 grams for males. On a few occasions it was possible to obtain 26 gram females for exact comparison with the 26 gram males. To simplify subsequent discussion, males or females weighing 10±1 grams are designated as small; those weighing 18±1 grams as medium; and males weighing 26±1 grams; or females weighing 24±1 grams as large. Injections were intraperitoneal except in the cases of alpha-naphthyl thiourea (ANTU) and histamine, for each of which it was desired to compare intravenous with intraperitoneal routes.

Following necessary dose range finding, work schedules were arranged so that a three-dose LD50 determination was carried out separately on males and females of each weight group on a single day. Additional determinations were made on subsequent days to the extent necessary to achieve the desired precision. Each day's data was calculated separately and tested for combinability with other days' results before final calculations were made. The method of Litchfield and Wilcoxon (1949) was used throughout. Because significant differences were found in the responsiveness of the two sexes for some drugs, all data was kept separated by sex. All dosages are expressed as micrograms or milligrams per animal.

Results

The calculated LD50 for each drug for each sex for each weight group was plotted against body weight. The extreme range of doses involved (0.12 µg to 57.7 mg) dictated the use of a logarithmic scale to facilitate comparisons. Since the desired ultimate comparison was between slopes of these lines, a logarithmic scale was also used for body weight, The data are presented graphically in the two accompanying figures. Figure 1 shows those cases where the relationship seems expressed by a straight line, while Figure 2 shows those where the relationship is some other function. The numerical data are presented in Table 1.

Discussion and Conclusions

It seems clear that in the great majority of cases tested there is a direct and approximately straight-line relationship between the logarithms of the LD50 and the body weight of the mouse. Sophisticated mathematical evaluation of these lines has not been made. However, inspection of the data and of the calculated slopes of the lines clearly indicates that the most common relationship is a direct, linear one characterized by a unity slope.

It is equally obvious that several cases are not characterized by such a relationship. There are various slopes and some lines are far from straight. Furthermore, it is apparent that route of injection does not consistently influence results. In the case of histamine; intraperitoneal and intravenous injections produced very similar relationships (in spite of somewhat different sensitivities), while in the case of ANTU the two routes

of injection gave quite different results. The influence of sex is also inconsistent as illustrated by the situation with hemicholinium-3 which is quite different from most other cases.

From the evidence presented, it seems inescapable that generalization with respect to the relationship between effective dose and body weight of the test organism is not justifiable. The character of the relationship varies in a way thus far not predictable with route of administration, identity of drug, and sex of animal. Furthermore, such an overall conclusion is the only way to reconcile the extreme variety of relationships which have previously been reported.

The practical application of these findings leads inevitably to the conclusion that there is no short cut by which to predict the magnitude of effect of a given dose of a drug in an individual test organism. To the clinician this is a considerable inconvenience. To the laboratory scientist it is also inconvenient, but a ray of hope can be held out. Some form of relationship exists in each case. Once it has been identified it can, with proper ~ution, be useful. Its suitability for application beyond the limits of a single test situation would be a fortunate coincidence - no more.

Summary

LD50s have been determined for tetrodotoxin, hemicholinium-3, d-tubocurarine, strychnine, α-naphthyl thiourea, 48-80 (a histamine liberator), picrotoxin, sodium fluoroacetate, McN-A-343 (a ganglionic stimulant), hexamethonium chloride, pentamethylene tetrazol, sodium pentobarbital, atropine,

histamine, and sodium barbital by intraperitoneal injection into small, medium, and large male and female mice. Values have also been determined for histamine and α-naphthyl thiourea by intravenous injection. An attempt has been made to describe the relationship of toxicity to body weight for each of these instances.

The most common dose to body weight relationship found is a direct linear one characterized by a slope of unity. However, a significant number of cases show a variety of other relationships, and preclude any overall generalization. Experimental evidence is provided to demonstrate that the character of the relationship may vary with drug identity, route of administration, and sex of the test organism. There is no reason to expect that species, strain, and the other factors known to modify the intensity of drug action will not also modify the relationship of effective dose to body weight. The influence of each of these factors is, however, not predictable on the basis of present knowledge.

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Table 1. Relationship of Toxicity[a] to Body Weight

Drug	Sex	Dose Units	SMALL (10±1 gm) LD50	SMALL (10±1 gm) Conf. Lim.	MEDIUM (18±1 gm) LD50	MEDIUM (18±1 gm) Conf. Lim.	LARGE (M 26±1 gm; F 24±1 gm) LD50	LARGE (M 26±1 gm; F 24±1 gm) Conf. Lim.	Slope[b] of Dose:Weight Regression Line Med/Sm	Lge/Med	Lge/Sm
Tetrodotoxin	M	µg	0.13	0.12-0.14	0.21	0.20-0.22	0.27	0.26-0.28	0.90	0.90	0.80
	F		0.12	0.11-0.13	0.21	0.20-0.22	0.26	0.25-0.27	0.97	0.93	0.90
Hemicholinium-3	M	µg	1.59	1.43-1.76	2.38	2.16-2.62	3.44	3.04-3.89	0.83	1.01	0.83
	F		1.58	1.41-1.77	2.43	2.15-2.75	1.80	1.62-2.00	0.86	0.51	0.44
d-Tubocurarine hydrochloride	M	µg	5.28	4.93-5.65	8.82	8.09-9.61	13.6	12.8-14.4	0.93	1.07	0.99
	F		5.04	4.75-5.34	8.50	7.94-9.10	11.0	10.4-11.7	0.93	0.98	0.91
Strychnine sulfate	M	µg	14.0	13.3-14.7	31.0	29.3-32.9	48.0	45.3-50.9	1.23	1.08	1.32
	F		15.1	14.5-15.7	30.0	28.0-32.1	41.9	39.2-44.8	1.11	1.05	1.15
α-Naphthyl thiourea (ANTU)	M	µg[c]	19.4	11.6-32.6	207	195-219	400	342-468	5.94	1.34	7.92
	F		26.5	18.3-38.4	262	243-283	514	463-571	5.50	1.36	7.46
	M	µg	57.0	49.0-67.0	207	186-230	396	374-420	2.01	1.32	2.66
	F		57.0	49.0-67.0	232	205-262	479	452-508	2.26	1.36	3.73
48-80	M	µg	56.3	53.6-59.1	85.5	82.2-88.9	104	100-108	0.84	0.85	0.71
	F		53.0	51.0-55.1	78.8	75.8-82.0	90.2	86.7-93.8	0.83	0.86	0.71
Picrotoxin	M	µg	87.6	83.4-92.0	209	201-217	290	279-302	1.33	0.96	1.27
	F		89.4	85.1-93.9	205	199-211	266	258-274	1.27	0.98	1.24
Sodium fluoroacetate	M	µg	150	138-164	246	226-268	281	258-306	0.91	0.79	0.72
	F		168	154-183	266	242-293	249	206-301	0.88	0.65	0.57
McN-A-343	M	µg	362	320-409	695	451-1070	922	887-959	1.07	0.92	0.98
	F		410	390-431	666	424-1046	825	793-858	0.90	0.93	0.84
Hexamethonium chloride	M	mg	0.95	0.90-1.00	1.60	1.51-1.70	1.89	1.77-2.02	0.93	0.82	0.77
	F		0.95	0.91-0.99	1.38	1.31-1.45	1.46	1.36-1.56	0.81	0.80	0.64

[a] Intraperitoneal injection except where indicated otherwise

[b] Ratio of LD50s divided by ratio of weights

[c] Intravenous injection

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Table 1. Relationship of Toxicity[a] to Body Weight (Cont'd)

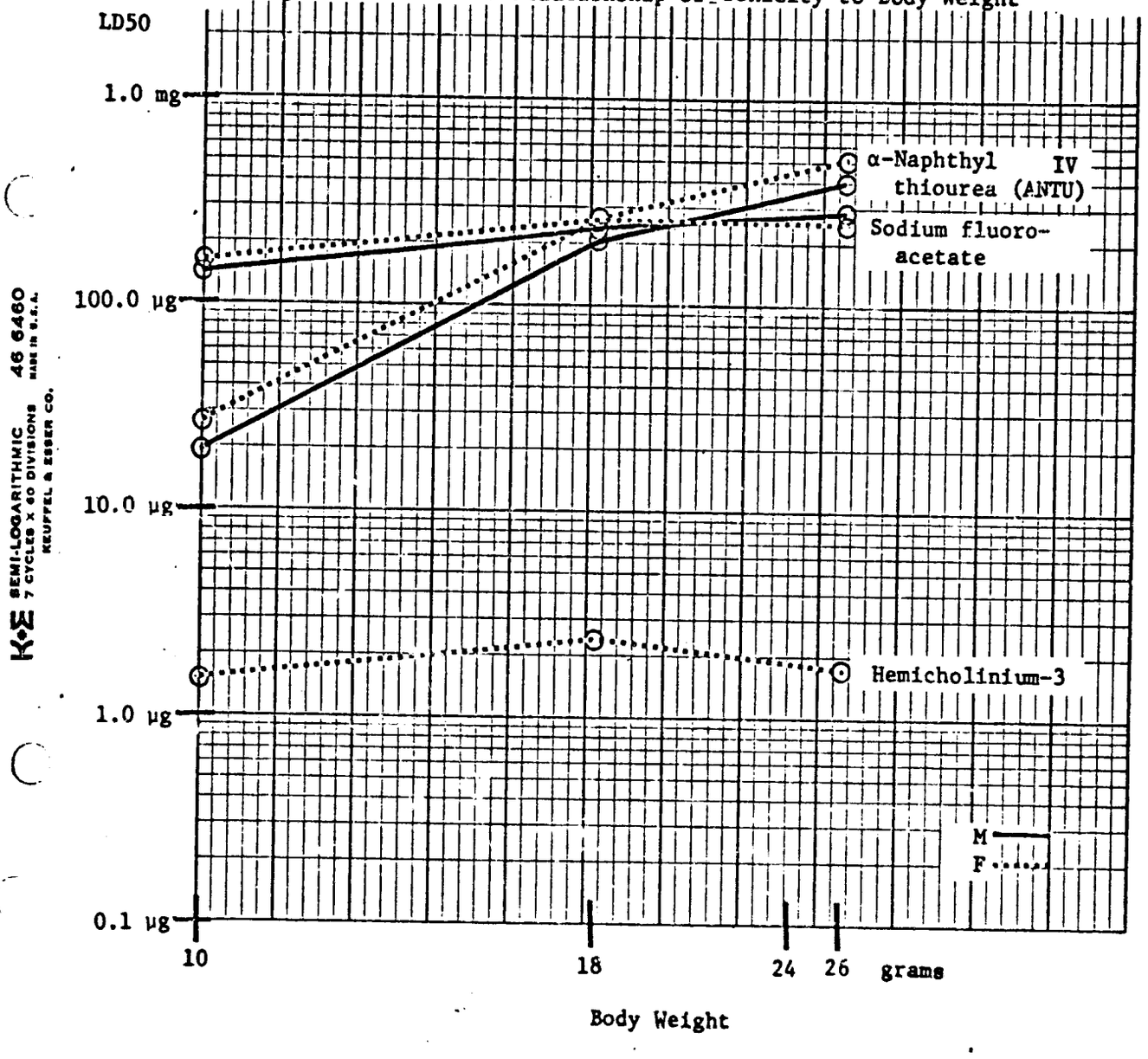
Drug	Sex	Dose Units	SMALL (10±1 gm) LD50	SMALL (10±1 gm) Conf. Lim.	MEDIUM (18±1 gm) LD50	MEDIUM (18±1 gm) Conf. Lim.	LARGE (M 26±1 gm;F 24± 1 gm) LD50	LARGE (M 26±1 gm;F 24± 1 gm) Conf. Lim.	Slope[b] of Dose:Weight Regression Line Med/Sm	Lge/Med	Lge/Sm
Pentamethylene tetrazol	M	mg	0.97	0.92-1.02	1.74	1.69-1.79	2.15	2.09-2.21	0.99	0.86	0.85
	F		1.02	0.61-1.71	1.62	1.57-1.67	1.77	1.70-1.84	0.88	0.83	0.73
Sodium pentobarbital	M	mg	1.13	1.10-1.16	2.05	1.99-2.11	3.00	2.91-3.09	1.01	1.01	1.02
	F		1.08	1.04-1.12	2.09	2.03-2.15	3.19	3.13-3.25	1.06	1.06	1.13
Atropine sulfate	M	mg	2.34	2.21-2.48	4.10	3.40-4.31	5.53	5.32-5.75	0.97	0.94	0.91
	F		2.14	2.02-2.27	3.71	3.50-3.93	4.79	4.56-5.03	0.96	0.90	0.86
Histamine diphosphate	M	mg[c]	4.32	3.72-5.01	7.20	6.49-7.99	10.3	9.36-11.3	0.93	0.99	0.92
	F		3.29	2.70-4.01	7.20	6.55-7.92	10.3	9.36-11.3	1.22	0.99	1.20
	M	mg	23.7	22.4-25.1	39.9	38.0-41.9	57.7	51.1-65.2	0.93	1.01	0.93
	F		24.5	23.1-26.0	36.7	34.6-38.9	47.8	43.5-52.6	0.83	0.90	0.75
Sodium barbital	M	mg	8.08	7.77-8.40	14.2	13.7-14.8	18.5	17.3-19.8	0.98	0.90	0.88
	F		7.89	7.66-8.13	13.6	13.1-14.1	18.3	17.6-19.0	0.96	0.94	0.89

[a] Intraperitoneal injection except where indicated otherwise

[b] Ratio of LD50s divided by ratio of weights

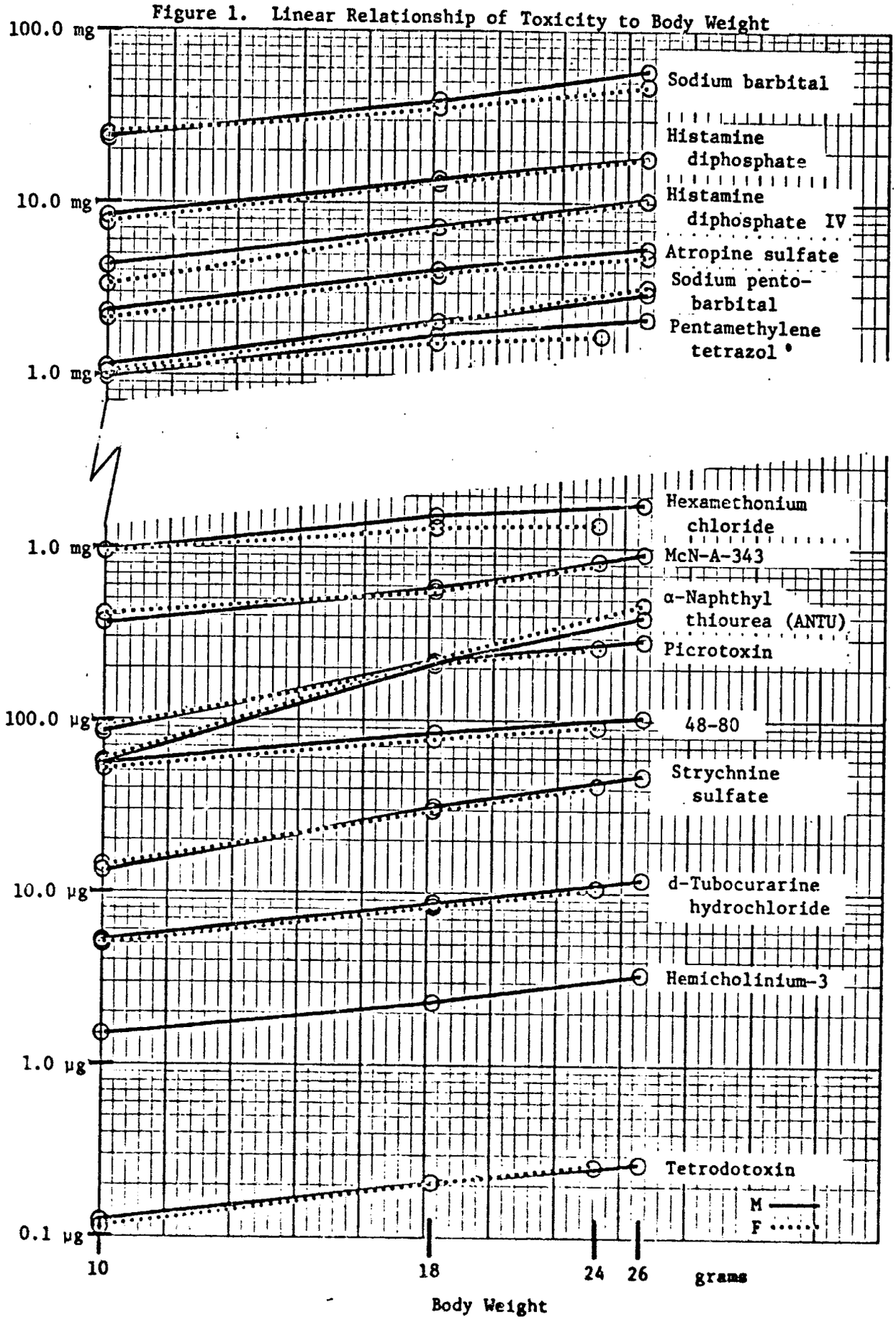
[c] Intravenous injection

Figure 2. Nonlinear Relationship of Toxicity to Body Weight



LD50

Figure 1. Linear Relationship of Toxicity to Body Weight



Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Bionetics Research Laboratories, Inc.
P.O. Box 26, Falls Church, Virginia 22046

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
--

3. REPORT TITLE
Relationship of Effective Dose to Body Weight (U)

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Final Report August 1964 to September 1967

5. AUTHOR(S) *(First name, middle initial, last name)*
E. Ross Hart, Ph.D.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1967	13	14

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
Contract No. DA-49-092-ARO-22	NA
b. PROJECT NO.	
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	NA

10. DISTRIBUTION STATEMENT
Each transmittal of this document outside the agencies of the U.S. Government must have prior approval of the DA, ARO, CRDLS

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
NA	Army Research Office

13. ABSTRACT

LD50s have been determined for tetrodotoxin, hemicholinium-3, d-tubocurarine, strychnine, α-naphthyl thiourea, 48-80 (a histamine liberator), picrotoxin, sodium fluoroacetate, McN-A-343 (a ganglionic stimulant), hexamethonium chloride, pentamethylene tetrazol, sodium pentobarbital, atropine, histamine, and sodium barbital by intraperitoneal injection into small, medium, and large male and female mice. Values have also been determined for histamine and α-naphthyl thiourea by intravenous injection. An attempt has been made to describe the relationship of toxicity to body weight for each of these instances.(U)

The most common dose to body weight relationship found is a direct linear one characterized by a slope of unity. However, a significant number of cases show a variety of other relationships, and preclude any overall generalization. Experimental evidence is provided to demonstrate that the character of the relationship may vary with drug identity, route of administration, and sex of the test organism. There is no reason to expect that species, strain, and the other factors known to modify the intensity of drug action will not also modify the relationship of effective dose to body weight. The influence of each of these factors is, however, not predictable on the basis of present knowledge.(U)

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Effective Dose Body Weight Mouse						

Security Classification

SUPPLEMENTARY

INFORMATION

IDENTIFICATION	FORMER STATEMENT	NEW STATEMENT	AUTHORITY
AD-823 546L Bionetics Research Labs., Inc., Falls Church, Va. Final rept. Aug 64-Sep 67. Sep 67 Contract DA-49-092-ARO-22	USGO: others to Army Research Office, Attn: CRDLS, Washington, D. C.	No limitation	OCRD, D/A ltr, 30 Oct 69