Imbibition and Extraction of Powdered Jalap.—Using belladonna root in No. 40 powder, Husa and Magid (1) found that imbibition of alcohol-water mixtures increased with decreasing alcohol content; similar results were obtained in the present study using jalap in No. 60 powder. As far as present results go, alcohol of U. S. P. strength and absolute alcohol seem to be the best solvents for the extraction of jalap resin; with more aqueous mixtures a greater proportion of inert extractive is removed along with the resin.

SUMMARY.

Alcohol, water and glycerin have been studied from the standpoint of swelling effects and rate of penetration, using jalap in the form of thin strips and blocks. Using a series of alcohol-water mixtures in a study of imbibition and extraction of powdered jalap, it was found that with increasing concentration of alcohol there is a decrease in imbibition and a decrease in the proportion of extraneous matter extracted along with the resins.

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THE APPLICATION OF STATISTICAL METHODS TO PHARMACEUTICAL RESEARCH. IV. METHODS OF RECORDING DRUG ACTION.

BY J. C. MUNCH[1] AND F. E. GARLOUGH.[2]

How much of a drug is required to produce a definite action? This question constantly arises in discussing the narcotic potency of a drug like cocaine, the relative soporific value of several barbiturates, the anesthetic concentration of ether, the cathartic dose of cascara, the lethal or the convulsant dose of strychnine. Official compendiums list the "doses" of drugs as a matter of convenience and of practicality. Many original articles dealing with quantitative measures of drug action, as well as compilations of toxic and lethal doses (9) give tables showing doses per animal or per kilo, which are withstood, which produce injury, which produce the desired type of response, or which produce death within stated time limits.

The general impression is regarding relationship of dose to effect results from the usual method of laboratory study. A series of doses of a product is given to animals and the effects observed. Too small a dose fails to produce discernible or detectable effects: this is a "subminimal" or "subliminal" quantity. As the dose is increased there is a change in the normal appearance of the test animal, which is attributed to the action of the drug. After showing the characteristic response for some time, the effect passes off and the animal recovers. The smallest concentration that produces such a response is often called the "minimum effective dose" (MED). Increases in dose cause greater intensity or prolongation of action until a quantity is reached that causes some of the test animals to die. This dose may be

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called the "maximum tolerated dose" (MTD) or the "minimum lethal dose" (MLD). With still further increases in dosage, further increases in the death rate are observed until a sufficient quantity is administered to produce death in all the test animals. This quantity may be known as the "absolute lethal dose" (ALD) or the "certain lethal dose" (CLD).

Probably as a means of convenient expression, the minimum lethal dose (MLD) has been used as a basis for recording the relative activity of different drugs under the same conditions, and of the same drug under different conditions. All workers are not interpreting MLD in the same manner. To some it means that dose which just produces death in one or more animals (usually of an unstated total number tested); to others it means the dose that kills all animals injected (one or more!); to others it means that dose that kills two-thirds, three-fourths or four-fifths of the test animals. It is obviously impossible to compare results obtained by different investigators under such conditions.

From the results and interpretations published in many reports, it would appear that some investigators believe that the administration of gradually increasing quantities of a drug produces no effect whatsoever, until suddenly the addition of the last increment causes an overwhelming response, and a passage from 0 per cent to 100 per cent of deaths of the injected animals. However, careful studies of drug action have shown that animals do not respond in this abrupt fashion. So long as the dose is below the effective dose on the most susceptible animal tested, no effect can be detected. As the dose is increased, the most susceptible animals react. There is a marked difference in susceptibility of various animals selected from the same colony, born and bred under ostensibly identical conditions. With increase in dosage, there is an increase in the percentage of animals showing a positive response, until finally that dose is reached that produces the characteristic effect in all the test animals. The relation between dose and effect has been found to follow an ogive or S-shaped curve. The reports by Shackell and co-workers (23, 24, 25) in 1923, appear to be the earliest pointing out the S-curve relationship. In a paper published in 1927, Trevan (26) developed the variability of animals into a blessing rather than a curse: that is, he developed the "standard curve" into a method of more exact measurement of activity, and showed that the form of the characteristic curve depended upon the combined effect of a large number of factors (sex, temperature, diet, etc., etc.). He confirmed earlier work (7), indicating the relationship between the number of animals used and the variability of the result obtained. He also pointed out that the "discrepant" or unexpected results should not be eliminated from the final average "except on the clearest evidence of a mistake in technique." "Even when the medial lethal dose remains constant, the preparation of a standard is necessary to correlate the work of different laboratories, and to eliminate the effect of variations in technique and the strain of animals in use."

Investigations (1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 17, 22) have shown that this curvilinear relationship seems to be a general index of animal response to drugs of various pharmacodynamic activities. Various special and rather involved mathematical procedures have been proposed by which the relation between dose and effect will approach linear, rather than curvilinear representation. In general, there is a very close approach to a linear relationship when doses are considered affecting between 25 and 75 per cent of the total animals used: in many instances

the range may be extended to the interval between 10 per cent and 90 per cent. No definite relationship has been established between the dose affecting 1 per cent and the dose affecting 100 per cent: in some instances the ratio is 2, in others 20. Since the curve most closely approaches a straight line for the dose affecting half of the test animals, Trevan proposed the "LD_{50}" dose as a basis of measurement. This has been substantiated in subsequent investigations, although comparisons can be made at any other level of activity, as long as the same intensity of response is measured for all the drugs compared.

In our studies on aconite, morphine, picrotoxin and strychnine, we have had an opportunity to investigate the possibilities of using the standard curve. Results may be measured for accuracy and correlation by methods recorded in previous papers in this series (16, 18). The customary method of expression of the LD_{50} or any other quantitative effect was incomplete because the number of animals giving this percentage response was unknown. This may be remedied by the use of a

TABLE I.—TOXICITY OF ACONITINE TO WHITE RATS.

Dose Mg./Kg.	Per Cent Killed. Subcutaneous.	Intraperitoneal.
0.05	..	0
0.06	..	0
0.07	..	44
0.075	..	33
0.08	0	52
0.09	..	61
0.10	8	84
0.11	..	87
0.125	35	96
0.15	47	100
0.175	77	...
0.20	91	...
0.22	100	...

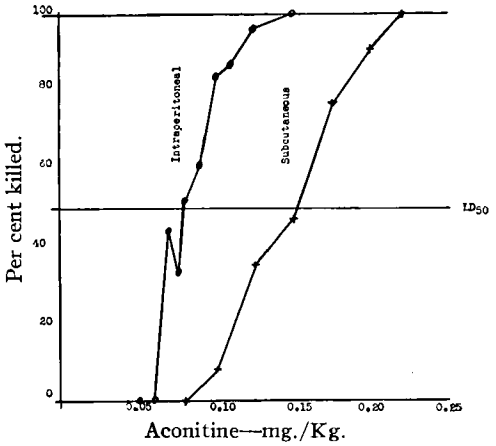

Fig. 1.—Toxicity of aconitine to white rats—mg./Kg.

subscript indicating the number of animals used preceding capital letters indicating the nature of the response desired, and followed by another subscript indicating the percentage of reacting animals taken as a desirable degree of drug effect. For example, if the subcutaneous injection of 0.06 mg. per Kg. of aconitine to guinea pigs killed 9 of 10 injected animals, this might be condensed to: aconitine, guinea pig subcutaneous, 0.06 $mk_{10}LD_{90\%}$. Now, if 0.04 mg. per Kg. killed 1 of 5 guinea pigs it would be recorded as: 0.04 $mk_5LD_{20\%}$. Similar expressions may be developed for anesthetic, narcotic, soporific, depressant or other types of drug response.

In studying the lethal dose of aconitine (21), a series of rats weighing between 150 and 200 Gm. were fed the same diet and stored under the same conditions. Subcutaneous or intraperitoneal injections were made, observing the usual technique. After it was established that subcutaneous injection of 0.08 mg. of aconitine per Kg. killed none of 25 rats, or 0 per cent, successive series of rats were injected with progressively larger doses up to 0.22 mg. per Kg. which killed 100 per cent. Consolidated results showing the doses and the percentage of animals killed are given in Table I and Fig. 1.

The U. S. P. guinea pig method for the bioassay of aconite requires that 0.060 mg. of aconitine per Kg. should kill 2 of 3 guinea pigs within 12 hours after subcutaneous injection. This would correspond to 0.06 mk_3LD_{67}. Compared at this level, the LD_{67} of aconitine for rats was 0.175 mg. per Kg. showing that approximately 3 times as large a dose of aconitine was required to kill rats as to kill guinea pigs.

In studying the action of picrotoxin upon mice following subcutaneous injections, it was found (20) that doses below 1.5 mg. per Kg. produced no detectable effect; doses between 2.0 and 3.5 mg. per Kg. produced convulsions; and doses between 3.0 and 3.5 mg. per Kg. produced death (Table II and Fig. 2). Plotting these results showed a convulsant zone extending on the one side into the non-convulsant, and on the other to the death zone. Bioassays of picrotoxin and its preparations could be made by simultaneous tests upon mice with a standard picrotoxin and the unknown product, to determine the $_5LD_{80}$%, and the activity could be expressed in terms of standard picrotoxin. In general, 5 mg. per Kg. of picrotoxin produced this effect, although variations in the susceptibility of the mouse colony required the use of doses ranging from 4 to 7 mg. per Kg. The CD_{50} was approximately 2 mk.; the LD_{50} 4 mk., or twice the CD.

TABLE II.—ACTION OF PICROTOXIN UPON MICE—SUBCUTANEOUS INJECTION.

Dose Mg./Kg.	Per Cent Showing. Convulsions.	Death.
1.5	0	0
2.0	54	0
2.5	80	0
3.0	96	35
3.5	100	22
4.0	100	29
4.5	100	23
5.0	100	69
5.5	100	100

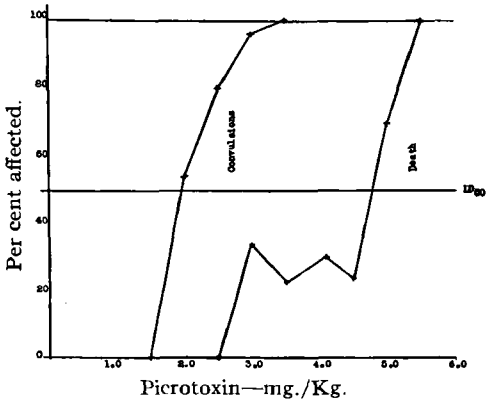

Fig. 2.—Action of picrotoxin on mice—subcutaneous injection.

TABLE III.—POSITIVE MOUSE TAIL RESPONSE OF MICE TO MORPHINE—SUBCUTANEOUS INJECTION.

Dose Mg./Kg.	Per Cent Showing Positive Response. Lab. A.	Lab. B.	Lab. C.
0.5	...	16	...
1.0	...	53	0
1.11	8	...	...
1.25	20	...	1
1.43	39	...	...
1.67	60	...	5
2.0	85	79	10
2.5	..	91	20
3.0	..	100	31
4.0	..	...	40
5.0	..	100	65
6.0	..	...	90
7.0	..	...	100

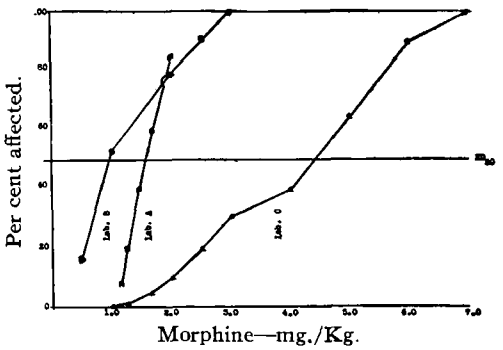

Fig. 3.—Positive reactions of mice to morphine—subcutaneous injection.

In studying the variations in susceptibility of mice to subcutaneous injections of morphine solutions (19), using the "tail curve" as an index of activity, tests were conducted independently in three different laboratories. Results obtained are shown in Table III and Fig. 3 and indicate typical S-curve responses. The differences in quantitative responses in different laboratories indicate the effects of various conditions that were not identical. For this reason it is obviously necessary to standardize the behavior of test mice. These great divergences in different laboratories indicate the futility of attempting to say that a particular dose of morphine is the smallest dose that will produce a typical curvature of the tail.

Our studies upon the lethal doses of strychnine administered subcutaneously, intraperitoneally, incorporated with food or administered by stomach tube to hundreds of ground squirrels, prairie dogs and rats have shown wide species variations. In tests by Moore, Spencer and Ward, under various working conditions, attempts have been made to determine the LD_{10}, LD_{50}, LD_{90} and LD_{100} upon animals deprived of food for 24 hours, then fed bait containing a known amount of strychnine, or injected with 0.5 per cent strychnine solution by stomach tube. In the control of noxious rodents, it is desired to obtain at least LD_{90} and it is ideal to hope for an LD_{100}, response. The data obtained on adult animals following oral administration of strychnine in the course of these investigations are given in Table IV and Fig. 4. It is obvious that there is a species difference between the Douglas ground squirrel and the Columbian ground squirrel. The former is much more susceptible to small doses, even though the L_{100} doses are practically identical. The different effects following changes in altitude are observed in the results obtained on rats. In Ward's experiments in Denver (27) at 5280 feet, rats were much more susceptible than litter mates that had been shipped to Portland, Oregon, held there for three months on the same food, and then injected with the same strychnine by Moore (27).

TABLE IV.—VARIATION IN ORAL LETHAL DOSE OF STRYCHNINE FOR RODENTS, DOSE IN MG./KG.

Animals.	Per Cent Killed. LD_{10}.	LD_{50}.	LD_{90}.	LD_{100}.
Ground squirrel (Douglas)	3.0	8.0	20	22.0
Ground squirrel (Columbian)	8.0	12.0	18	22.5
Prairie Dog (Zuni)	3.0	4.0	5	7.0
Rats—Denver	5.0	7.5	..	10.0
Rats—Portland	7.5	9.0	..	12.5

increase in altitude. The LD_{100} was 22.5 mk. at 2600 feet, and decreased approximately 1 mk. for every 500-foot elevation to an LD_{100} of 15 mk. at 6500 feet.

In studies upon Zuni prairie dogs, the season has been found to affect the toxicity, possibly because of the differ-

Tests by Moore on 482 Columbian ground squirrels showed definite variations in susceptibility to strychnine with

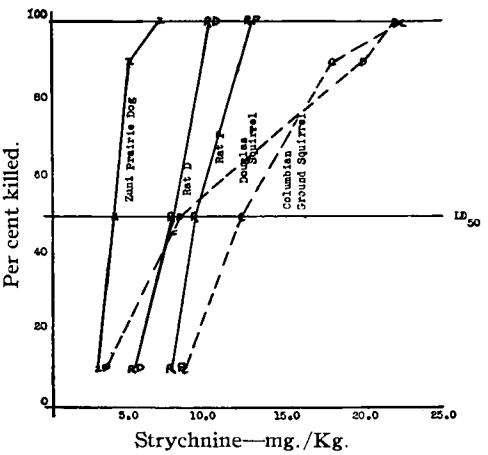

Fig. 4.—Toxicity of strychnine to rodents—oral administration—mg./Kg.

ence in tannin content of the food. In the spring the LD_{100} was 7 mk., in the fall, 4 mk. In these various tests, at various altitudes, and with animals on various diets, however, the response to strychnine has been found to follow an S-shape curve, whether considering the convulsant or the fatal dose.

CONCLUSIONS.

1. The standard curve is useful in recording the relation between dose and effect.

2. Most accurate results are obtained in determining those doses that produce desired responses in half of the animals tested.

3. By using a subscript before the statement of effect to indicate the number of animals employed, and another after it to indicate the degree of response used as a criterion ($_{n}ED_{\%}$), drug action may be quantitatively recorded with maximum convenience and minimum labor.

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CYANIDE POISONING AND ITS TREATMENT.*[1,2]

BY K. K. CHEN, CHARLES L. ROSE AND G. H. A. CLOWES.

MORTALITY STATISTICS.

Cyanide poisoning is less frequent than mercury or strychnine poisoning, although during recent years the death rate from cyanide poisoning has been increasing. In Table I it will be seen that in the United States Registration Area there were 134 deaths from cyanide poisoning in 1930, and 243, 408 and 416 in 1931,

TABLE I.—MORTALITY STATISTICS FROM CYANIDE POISONING.**

Locality.	Population (1930 Census).	Total Number of Deaths. 1922.	1923.	1926.	1927.	1928.	1929.	1930.	1931.	1932.	1933.	1934.
U. S. Registration Area	122,775,046	102	113	79	84	108	141	134	243	408	416	..*
New York City	6,930,446	...*	...*	14	11	23	21	34	16	42	35	27
Chicago	3,376,438	...*	...*	3	8	14	4	12	16	25	17	6
Philadelphia	1,950,961	...*	...*	2	2	1	3	0	3	4	3	..*
Detroit	1,568,662	...*	...*	2	0	6	2	7	5	15	11	5
Los Angeles	1,238,048	...*	...*	12	14	19	14	18	23	34	33	25
Cleveland	900,429	...*	...*	1	0	3	2	0	1	4	6	1
Saint Louis	821,960	...*	...*	6	2	6	2	7	9	8	6	2
Baltimore	804,874	...*	...*	1	2	1	5	3	2	5	1	1
Boston	781,188	...*	...*	0	6	2	0	3	8	7	3	8
Pittsburgh	669,817	...*	...*	1	1	2	1	2	5	3	1	1
San Francisco	634,394	...*	...*	9	9	8	10	11	12	21	22	23

* Data not available.

** In the compilation of this Table, we were greatly assisted by Dr. T. F. Murphy, Chief Statistician for Vital Statistics, Bureau of Census, Department of Commerce, Washington, who generously turned over to us all the data which he has collected with meticulous care. Our indebtedness must also be acknowledged to Doctors J. C. Geiger, Director of the Department of Public Health, City and County of San Francisco; Herman N. Bundesen, President of the Board

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[1] From the Lilly Research Laboratories, Indianapolis.

[2] Scientific Section, A. PH. A., Portland meeting, 1935.