toxicity of this drug. Moreover, in view of two recent reports which indicate that magnitude of prior social experience (15) and quantity of body contact (16) represent important factors influencing the expression of the amphetamine aggregation effect, it is evident that this phenomenon represents the product of a complex interaction between several environmental, genetic, and pharmacological factors.

SUMMARY

A series of experiments was designed to test the hypothesis that hyperthermia is a major factor contributing to the increased toxicity of amphetamine observed in aggregated *versus* isolated mice. Incidence of lethality and rectal temperatures were recorded hourly over a 3-hour interval following the intraperitoneal administration of 100 mg./Kg. amphetamine to animals placed in either an aggregated (3 mice per 7 × 7 × 7.5 cm. cage) or isolated environment. The effect of pretreatment with several agents known to influence body temperature (chlorpromazine, acetylsalicylic acid, and phenoxybenzamine) was also determined. On the basis of the results obtained the following conclusions appear to be justified:

Although amphetamine is definitely more toxic in grouped animals (aggregation effect), the degree of hyperthermia induced may be essentially the same for both aggregated (40.6°) and isolated (40.2°) mice.

Drugs which have the ability to protect animals against the occurrence of the amphetamine aggregation phenomenon, *e.g.*, chlorpromazine, may do so at dose levels which are not hypothermic *per se* and which do not alter amphetamine-induced hyperthermia.

Phenoxybenzamine, which has the ability to depress markedly the hyperthermic response to amphetamine, does not consistently abolish the lethality of this drug in an isolated environment.

Pretreatment with acetylsalicylic acid using a dose level which decreases the magnitude of amphetamine-induced hyperthermia significantly increases its lethality.

A generalization indicating a causal relationship between hyperthermia and the amphetamine aggregation phenomenon may be unwarranted.

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Spectrophotometric Method for the Assay of Individual Nitroglycerin Tablets

By FREDERICK K. BELL

A spectrophotometric procedure for the microdetermination of glyceryl trinitrate through alkaline hydrolysis and subsequent determination of the nitrite formed through diazotization and coupling has been devised which avoids the serious interference in the presence of lactose. The principal feature of the method is based on the substitution of strontium hydroxide for the usual sodium hydroxide as the hydrolyzing alkali. Presumably the formation of the strontium salt of lactose removes this carbohydrate from the field of interference. The results of more than 500 assays of individual tablets of nitroglycerin representing four different sources of manufacture and five different dosages are reported.

Probably because of its high degree of sensitivity and relative simplicity, a preferred method for the microdetermination of glyceryl trinitrate is the one based on the alkaline hydrolysis (1) of the nitrate ester and determination of the nitrite formed through the customary diazotization and coupling reaction and subsequent spectrophotometric measurement of the dye formed. An obvious application of this procedure would be the assay of individual nitroglycerin tablets in which suitable aliquots of a simple aqueous solution of the tablet would be subjected to the procedure.

However, it is known that lactose, which appears to be the excipient of preference in the manufacture of these tablets and is present in gross excess, seriously interferes with this analytical procedure (2). In a consideration of possible methods of avoiding this source of inter-

Received July 22, 1963, from the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore.
Accepted for publication October 8, 1963.

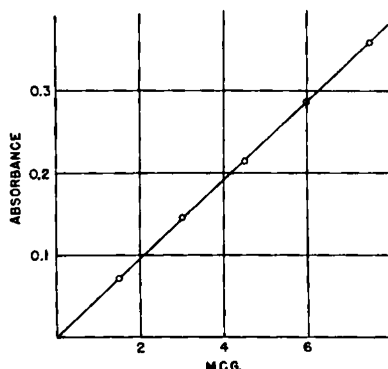

Fig. 1.—Standard recovery curve for glyceryl trinitrate.

sources of manufacture and in a number of different dosages.

MATERIALS

The assay values reported in this paper were obtained on commercial tablets purchased through the usual channels in bottles of 100 which originated from the following pharmaceutical firms: Abbott Laboratories, Eli Lilly and Co., Merck Sharp and Dohme, and Parke, Davis and Co. The dosage range included the following values: 1/100, 1/150, 1/200, 1/250, and 1/400 gr.[1]

The following reagents are required: procaine hydrochloride, 3 mg./ml.; hydrochloric acid, 4 *N*; *N*-(1 naphthyl) ethylenediamine dihydrochloride, 1 mg./ml.; and strontium hydroxide, octahydrate, 1 Gm./100 ml. It was somewhat disappointing that apparently the latter material is no longer readily available as a reagent chemical. The salt used in these studies was obtained from K & K Laboratories, Inc., Jamaica, N. Y., and the purity was indicated as 95–99%. Although appreciable turbidity in the 1% solution did not interfere with the procedure, the standard practice was to make up the day's requirement on the preceding day, thus providing a clear supernatant fluid for use in the assay. More prolonged storage of the solution was avoided.

TABLE I.—TYPICAL ASSAY DATA SHEET[a]

Tablet	Absorbance Observed	Absorbance Av.	Absorbance Corrected	Nitroglycerin Found, mcg.	Nitroglycerin Calcd., mg./Tablet
Control	0.016 0.018	0.017	...	...	...
1	0.275 0.283	0.279	0.262	5.5	0.69
2	0.292 0.291	0.292	0.275	5.8	0.73
3	0.262 0.258	0.260	0.243	5.1	0.64
4	0.214 0.211	0.213	0.196	4.1	0.51
5	0.252 0.255	0.254	0.237	5.0	0.63
6	0.275 0.274	0.275	0.258	5.4	0.68
7	0.264 0.265	0.265	0.248	5.2	0.65
8	0.280 0.280	0.280	0.263	5.5	0.69
9	0.290 0.298	0.294	0.277	5.8	0.73
10	0.276 0.277	0.277	0.260	5.45	0.68
				Av.	0.66

[a] Tablet X, dosage 1/100 gr., 0.2-ml. aliquots.

ference, the thought occurred that advantage might be taken of the well known fact that some carbohydrates, including lactose, form compounds with metallic ions, especially of the alkaline earth group. The stability of such a compound might be sufficient to reduce greatly or remove completely the lactose from the field of interference. Early preliminary experiments using barium hydroxide as the hydrolyzing alkali instead of the usual sodium hydroxide showed immediate promise. However, as the work progressed, strontium hydroxide was found to be considerably more efficient, both as the hydrolyzing alkali and as a supressor of the lactose interference.

This communication gives a detailed description of the method which has been devised and records the results obtained in the assay of commercial nitroglycerin tablets of four different

METHOD

The assay experiment was set up to include the assay of ten individual tablets, the average value of which was taken as the final assay value for the tablet. The procedure is as follows.

Place one tablet in each of ten 50-ml. glass-stoppered centrifuge tubes (Pyrex No. 8424). The tablet may be crushed with a glass rod. After adding exactly 25 ml. of distilled water to each tube, stopper and shake vigorously by hand for some

[1] Grateful acknowledgment is made to these firms for supplying tablets for the preliminary studies.

seconds to complete solution. Place the ten tubes in a shaking machine and shake vigorously for 5 minutes. An appreciable turbidity in the solution at this point is, of itself, of no significance since the mixture will undergo at least a tenfold dilution before spectrophotometric analysis. Transfer duplicate aliquots from each tube to a pair of 15-ml. graduated glass-stoppered centrifuge tubes. The schedule of aliquot volume with respect to tablet dosage is as follows: 0.2 ml. for 1/100 gr., 0.3 ml. for 1/150 gr., 0.4 ml. for 1/200 gr., 0.5 ml. for 1/250 gr., and 0.8 ml. for 1/400 gr. Distilled water is added to each of the 20 tubes to the 1-ml. mark, and 1 ml. of water is added to two additional tubes which serve as controls. From this point on, the experiment should proceed at reasonable speed without interruption. Add to each of the tubes 4 ml. of 1% strontium hydroxide. Stopper and mix by inversion. The lightly stoppered 22 tubes are then placed in a suitable rack which is then suspended in a water bath at 50 ± 0.2° so that the water level is approximately at the 10-ml. mark of the tubes. After 15 minutes the

Table II.—Summary of Assay Results[a]

Tablet	Dosage, gr.	Assay No.	Individual Tablet Assay Values, mg. × 100[b]										Av. mg.
			1	2	3	4	5	6	7	8	9	10	
A	1/100	1	69	55	80*	60	66	59	60	68	63	56	0.64
		2	67	58	62	59	66	75*	69	52	62	51*	0.62
		3	63	58	68	51*	70	63	50*	69	64	63	0.62
		4	55	54	66	76*	63	65	63	55	69	58	0.62
		5	68	68	69	55	66	54	63	51*	50*	68	0.61
B	1/100	6	75*	68	66	63	70	72	63	50*	58	65	0.65
		7	63	68	71	58	59	86*	64	75*	73	70	0.69
		8	74*	76*	67	50*	63	73	87*	73	69	59	0.69
		9	61	64	69	58	74*	78*	63	57	62	65	0.65
		10	65	48*	67	69	73	65	70	63	63	73	0.66
C	1/100	11	52	69	65	65	73	65	65	74	61	66	0.66
		12	63	65	63	67	74*	67	70	78*	63	70	0.68
		13	66	66	71	58	65	71	64	69	69	64	0.66
		14	59	68	62	69	67	69	68	68	63	71	0.66
		15	68	75*	66	71	73	66	66	75*	66	71	0.70
D	1/150	16	38	39	37	35	45	35	39	39	34	41	0.38
		17	41	39	44	43	38	38	38	43	46	42	0.41
		18	45	39	31*	39	40	37	33*	38	42	42	0.39
		19	41	42	47	41	35	47	39	43	49*	40	0.42
		20	49*	38	38	40	39	32	39	36	37	45	0.39
E	1/150	21	49*	45	46	44	47	50*	44	38	41	45	0.45
		22	39	35	48	37	49*	50*	44	42	42	44	0.43
		23	47	44	45	47	40	47	40	37	42	44	0.43
		24	47	43	44	43	50*	42	45	42	50*	50*	0.46
		25	40	41	47	43	41	48	39	45	47	43	0.43
F	1/150	26	40	45	42	50*	45	51*	47	42	36	42	0.44
		27	47	47	47	39	49*	42	42	47	48	47	0.46
		28	42	44	47	44	44	46	45	47	50*	50*	0.46
		29	33*	39	42	46	45	49*	47	44	42	44	0.43
		30	47	49*	47	46	40	46	44	43	45	49*	0.46
G	1/200	31	34	35	31	25*	34	28	28	35	40	32	0.32
		32	27	28	26	26	23*	33	27	33	36	29	0.29
		33	35	28	24*	22*	31	39*	27	35	23*	36	0.30
		34	35	21*	24*	41*	34	23*	28	28	35	33	0.30
		35	24*	48*	39*	35	35	24*	33	32	28	31	0.33
H	1/200	36	25*	29	25*	28	27	26	25*	28	28	28	0.27
		37	25*	27	29	26	29	29	26	26	27	29	0.27
		38	29	26	26	28	29	30	26	25*	25*	28	0.27
		39	29	31	29	28	28	28	28	25*	28	27	0.28
		40	25*	30	29	27	30	28	27	27	28	29	0.28
I	1/200	41	26	32	33	39*	36	38*	29	37*	30	34	0.33
		42	26	39*	35	38*	36	36	41*	37*	38*	39*	0.37
		43	35	31	40*	40*	26	29	38*	41*	27	30	0.34
		44	39*	34	36	37*	32	34	34	28	36	26	0.34
		45	31	31	41*	34	29	30	36	41*	41*	37*	0.35
J	1/250	46	24	27	24	24	25	25	21	23	23	26	0.24
		47	25	25	24	26	25	23	21	25	24	24	0.24
		48	21	25	25	23	24	24	24	25	21	24	0.24
		49	23	22	27	26	24	23	22	24	25	26	0.24
		50	28	21	25	26	25	22	22	24	22	27	0.24
K	1/400	51	19*	20*	18	18	18	12*	19*	21*	19*	15	0.18
		52	19*	16	18	20*	15	18	16	20*	21*	18	0.18
		53	15	16	18	17	17	19*	17	14	18	15	0.17
		54	20*	18	19*	21*	18	14	20*	17	14	20*	0.18
		55	21*	16	21*	20*	18	13	20*	18	14	18	0.18

[a] The tablets were carefully removed at random from original unopened bottles of 100. Visibly defective tablets were discarded when encountered. It appears reasonable to assume that the physical condition of these tablets at the time of assay approximates that which prevails at the time the patient receives them from the pharmacist. Individual weights of the tablets were not determined and therefore this factor, which is of considerable importance to the tablet manufacturer, is not included in the data. [b] Assay values with an asterisk indicate failure to meet U.S.P. requirements.

rack is removed from the bath, then immersed in cold tap water for approximately 5 minutes. The contents of each tube are treated in the following consecutive steps, mixing being accomplished after each step by inversion of the stoppered tube: (*a*) add 1 ml. procaine hydrochloride, 3 mg./ml.; (*b*) add 1 ml. hydrochloric acid, 4 *N;* (*c*) add 1 ml. *N*-(1-naphthyl)-ethylenediamine dihydrochloride, 1 mg./ml., and distilled water to the 10-ml. mark.

Full color development is attained in approximately 5 minutes, after which time the absorbance of each of the 22 solutions is measured with a Beckman model DU spectrophotometer at a wavelength setting of 550 mμ and a slit width of approximately 0.025 mm. Absorption cells having a light path of 1 cm. were used; distilled water served as the reference standard. The paired values are averaged and then corrected for the control value. The corrected values are then converted into terms of nitroglycerin with a standard recovery curve.

The preparation of the standard recovery curve using the procedure detailed above is quite similar to that described in an earlier communication (1). A typical curve is shown in Fig. 1. The reproducibility is very satisfactory.

A typical assay data sheet is shown in Table I and a summarized tabulation of all the tablets assayed in this study is shown in Table II. The ten-tablet assay, as described, requires several hours in the hands of one operator. An obvious modification of the procedure is a multiple-tablet composite assay in which ten tablets are dissolved in 250 ml. of water and aliquots of the proper volume are subjected to analysis. Using triplicate aliquots, such an assay can be carried out in less than 1 hour. The results obtained from a considerable number of such assays indicate the method is quite satisfactory. There is no reason why this method could not be extended to the case of 20 tablets in 500 ml. water or even 40 tablets in 1 L. if desired.

DISCUSSION

Concerning lactose interference, recovery experiments indicated that 5 mcg. of nitroglycerin can be satisfactorily determined in the presence of 1.5 mg. of lactose. This lactose-nitroglycerin ratio of 300:1 is considerably in excess of the maximum value encountered in this study which, of course, would be in the case of the 1/400-gr. tablets. The same observation holds for β-lactose. Sucrose, in similar amount, was without effect, which is in accord with the observations of Hansen (3).

If the procaine was not added before acidification in the assay procedure following the alkaline hydrolysis step, erratic results were obtained. This indicates that at least an important part of the lactose interference takes place at the diazotization step and that the strontium salt of lactose is of sufficient stability to suppress this interference with the diazotization reaction which probably takes place very rapidly.

In considering the data presented in Table II, it is quite obvious that a rigid quantitive interpretation is not permissible, since, if for no other reason, there are probably at least five different samples of glyceryl trinitrate involved in this study. If, however, the validity of the method is accepted, then the assay data reveal some interesting information.

First, it will be noted that the average assay value for each of the 55 assay experiments is within the limits required by the U.S.P. XVI, *i.e.*, within 80 and 112% of the labeled dosage. Furthermore, there is good agreement among the five average values obtained for each type of tablet. This indicates, from this limited study, that the ten-tablet procedure might be adequate to establish the assay value for a given tablet preparation. It is to be noted also that the inherent accuracy of the method increases as the tablet dosage decreases.

Individual tablet assay values which are starred in Table II indicate failure to meet U.S.P. requirements. Among the 550 assays listed there are 101 such values, of which 78 show excessive dosage and 23 fall below the requirements.

The assay method presented in this study should be of immediate interest to the control laboratory for the manufacture of nitroglycerin tablets. It provides information concerning the uniformity of the tablet dosage and also a rapid (ten-tablet composite procedure) assay procedure. Based on a knowledge of complete composition of the tablet, rigid controls could be established and the standard recovery curve could be prepared from the sample of nitroglycerin that is used in the preparation of the tablets.

To determine whatever merit the procedure may have as an official assay method would doubtless require considerable collaborative study. A reference standard preparation of glyceryl trinitrate would probably be required and, in this connection, a simple aqueous solution of the nitrate ester (1 mg./ml.) was surprisingly stable over a period of many months. However, Hansen (4) reports that an aqueous solution of 0.12 mg./ml. showed a 10% loss in potency after 10.5 months' storage.

Additional study will also be required to determine if the method has application to other pharmaceutical preparations containing nitroglycerin.

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