Figure 3 shows the relationship between collapse pressure and vinyl acetate content. The collapse pressure of pure polyvinyl acetate (10) is included for comparison. Increasing the vinyl acetate content results in higher collapse pressure. It is apparent that some interaction occurs between vinylpyrrolidone and vinyl acetate residues, since the collapse pressure of the 335 copolymer is higher than that of pure polyvinyl acetate.

Relation between Monolayers and Free Films—Previous work showed that certain properties of monolayers may be related to those of free films (5, 6). Collapse pressure in a group of related polymers is a measure of "hydrophobicity." A similar relation was found with the materials studied here. As shown in Fig. 4, moisture sorption in free films is inversely proportional to monolayer collapse pressure.

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Influence of Drug Particle Size after Intramuscular Dosage of Phenobarbital to Dogs

L. G. MILLER and J. H. FINCHER

Abstract □ Phenobarbital suspensions containing drug particles of different size ranges were separately administered, intramuscularly, to beagle dogs. The particle size of the dose affected the blood drug level curves. A comparison of the areas under the curves with the respective particle sizes indicated that particle size influenced the biological availability of phenobarbital. Data obtained suggest that by controlling the drug particle size, one may be able to regulate the duration of action of phenobarbital.

Keyphrases □ Particle size, drugs—effect on intramuscular administration of phenobarbital, beagle dogs □ Phenobarbital, intramuscular administration—effect of particle size on blood levels and bioavailability, beagle dogs □ Blood levels, dogs—effect of phenobarbital particle size after intramuscular administration □ Bioavailability—effect of phenobarbital particle size, intramuscular administration, beagle dogs

Within the last 15 years, it has become increasingly apparent that the biological availability of drugs can be altered considerably by their dosage forms and their physical properties. Particle-size influences on drug availability constitute an important part of pharmaceutical technology (1). Most reported studies to ascertain the influence of drug particle size were conducted with oral dosage forms, and only a few of these studies treated its effect in parenteral forms (2). Drugs in the GI tract are subjected to varying degrees of agitation and fluid composition; however, at intramuscular injection sites the agitation intensity is very low and the fluid composition should remain relatively constant. The intramuscular route may, therefore, be more useful in studying the influence of drug particle size. Reported herein are preliminary studies to determine the effects of drug particle size by intramuscular injection.

EXPERIMENTAL

Phenobarbital USP and sodium phenobarbital USP were utilized in these studies. The purities of the two drugs, based on dry weight, were determined by USP methods to be approximately 100% for the phenobarbital crystals and 92% for the sodium phenobarbital granules.

Twenty-five healthy, male, beagle dogs of approximately the same age and weighing between 10 and 15 kg. were the test subjects. From this group, three sets of eight were selected and alternated so that the same animal was not used for two successive experiments. The dogs were fasted for 18 hr. prior to use, but free intake of water was allowed. Intravenous injections were given in the cephalic vein of one foreleg, while the intramuscular injections were given in the gluteus medius; blood samples were drawn from the cephalic vein of the foreleg not used for the intravenous injection.

The particles to be studied were prepared by precipitating free phenobarbital from aqueous solutions of sodium phenobarbital with a 0.5 *N* hydrochloric acid solution. This procedure was done in an alcohol–dry ice bath at −10° under varying degrees of agitation and precipitation rates. A gross analysis of the crystals was made using a microscope, and the particle-size distribution was determined with a Coulter counter[1] (Fig. 1).

[1] Model B, Coulter Electronics Industrial Division, Chicago, IL 60614

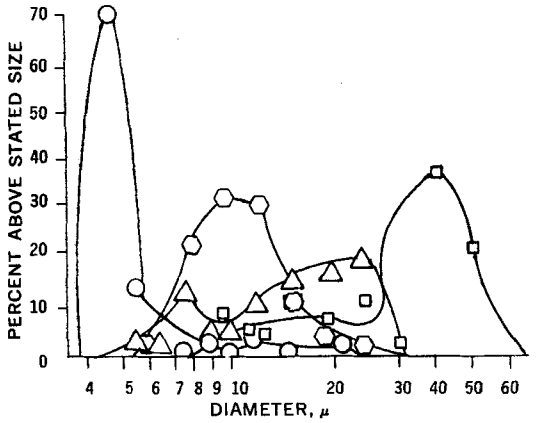

Figure 1—*Differential particle-size distributions of phenobarbital as analyzed by the Coulter counter. Key: ○, Sample P-4, mean diameter 6.63 μ; □, Sample P-5, mean diameter 29.96 μ; △, Sample P-6, mean diameter 17.16 μ; and ⬡, Sample P-7, mean diameter 10.68 μ.*

The parenteral solutions were made by dissolving an amount of sodium phenobarbital equivalent to 500 mg. of the free acid in a 5-ml. disposable syringe with 3 ml. of distilled water. The solutions were not made until just prior to the time of injection to reduce the degree of drug degradation.

Parenteral suspensions were prepared by weighing 500 mg. of the recrystallized phenobarbital sample, mixing it with 1% Marasperse CB[2], and placing it in a 5-ml. syringe. Distilled water was drawn into the syringe, and the suspension was shaken by hand until it was as uniform as possible. Parenteral suspensions were administered intramuscularly through a 16- or 18-gauge needle.

Sample assays were performed using a variation of Goldbaum's UV spectrophotometric method (3). The values were derived from a standard curve plotted from known concentrations of phenobarbital in 0.5 *N* sodium hydroxide assayed *versus* a sodium hydroxide blank in a grating spectrophotometer[3].

Each milliliter of a blood sample was placed in 0.2 ml. of a sodium citrate solution (4 g./100 ml.). Blood samples were subjected to a chloroform and sodium hydroxide extraction as follows. One- and two-tenths milliliter of blood (citrated) was pipeted into a 60-ml. separator containing 30 ml. of reagent chloroform. The separator was shaken by hand for 1 min., and the phases were allowed to separate for 1 min. A 25-ml. fraction of the chloroform was drawn through a dry filter paper into a graduate and then placed in a second separator containing 5.0 ml. of 0.5 *N* sodium hydroxide. After 1 min. of shaking and 1 min. of settling, the chloroform portion was drawn from the separator and discarded. The aqueous solution phase was decanted into a 5-ml. centrifuge tube and centrifuged for 1 min. to separate the remaining chloroform fraction. A 2.5-ml. sample of the clear supernatant was pipeted into a clean, dry cell[4] and analyzed against the sodium hydroxide blank. Several blood blanks were assayed by this procedure, and an average differential was used to determine the unknown concentration less the background.

Mean blood levels were determined after separate administrations of intravenous and intramuscular solutions, and the data were used as controls to ascertain the extent and rate of availability from suspensions. Blood samples were collected at intervals of 30 min. and 1, 3, 6, 9, 24, and 30 hr. Suspensions having mean particle diameters of 6.6, 10.68, 17.16, and 29.96 μ were given intramuscularly, and blood samples were collected in the same manner as described previously. The blood samples were kept cold by either ice coolers or refrigeration until the time of the assay.

To determine if the injected particle had any adverse effects on the muscle tissue or if tissue encapsulation had occurred, the site of injection was marked on one dog and examined by surgical procedures 72 hr. after administration of the larger particle suspension. The attending veterinarian conducted this examination, and his report was that the tissue at the site of injection remained normal and gave no indication of encapsulation (4).

[2] American Can Co., Chemical Products Sales, Neenah, Wis.
[3] Beckman DB-G, Beckman Instruments, Inc., Fullerton, CA 80566
[4] Beckman DU.

RESULTS AND DISCUSSION

The individual blood levels obtained after intramuscular administration of the various phenobarbital particle distributions are reported in Table I. Also reported in Table I are mean blood levels and the estimated standard deviation. The mean blood levels were plotted as a function of time (Fig. 2) for ease of comparison. It is obvious from Fig. 2 that the rates of availability of all intramuscular suspensions were slower than that of the intramuscular solution, and it is also apparent that the larger particle suspensions were available to a lesser extent than the smaller particle suspensions in a 30-hr. interval.

The areas under the blood level curves are a function of the biological availability of the total dose from each drug system (5). If the area is calculated for a definite time interval and if the blood concentration curve is an inexact differential depending upon absorption rates of the particles, then a comparison of the areas with the respective particle sizes is a comparative measure of the absorption rates of the particles.

By assuming that this hypothesis holds true, an examination of the 30-hr. data reveals that as the particle size increases the area under the blood level curve decreases (Table II). A semilog plot of the percent of area under the intravenous blood level curve as a function of particle diameter in microns yielded a linear expression.

Since surface area is proportional to diameter[2] (d^2) and since a linear plot of relative availability (as indicated by percent of intravenous dose) *versus* d^2 was not linear, the possibility that relative availability would be a direct function of surface area of the sample was not evident. Due to the nature of the dosage unit in the injection site, one would expect the availability to be a function of

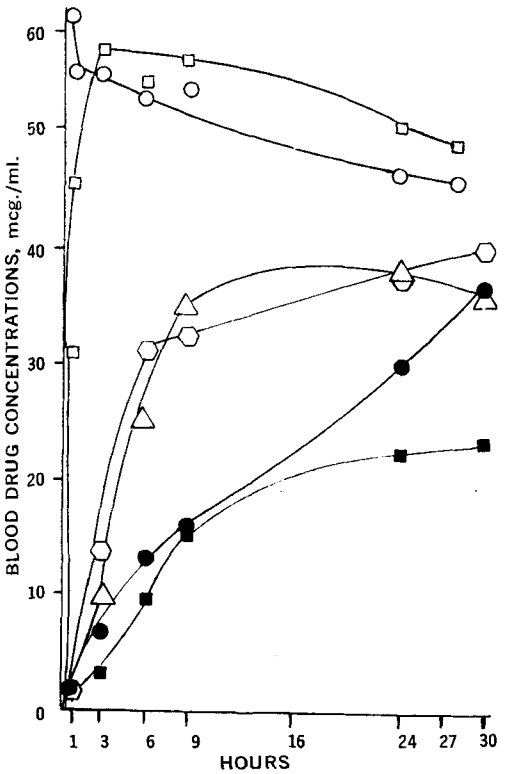

Figure 2—*Mean blood levels of phenobarbital in dogs as a function of time in hours after administration. Key: ○, intravenous solution of sodium phenobarbital equivalent to 500 mg. of free acid; □, intramuscular solution of sodium phenobarbital equivalent to 500 mg. of free acid; △, intramuscular suspension of Sample P-4 (6.63 μ); ■, intramuscular suspension of sample P-5 (29.96 μ); ●, intramuscular suspension of Sample P-6 (17.16 μ); and ⬡, intramuscular suspension of Sample P-7 (10.68 μ), each suspension containing 500 mg. of the free acid.*

Table I—Individual Blood Levels (Micrograms per Milliliter) Obtained at the Stated Times after Intramuscular Administration of the Various Phenobarbital Particle Distributions[a]

Sample	Trial	Hours: 1	3	6	9	24	27	30
		Blood Levels, mcg./ml.						
P-4 (6.63 μ)	1	6.0	26.4	38.4	58.4	49.8	58.8	48.3
	2	0.0	7.2	24.0	36.0	43.2	66.0	24.0
	3	6.6	20.4	53.4	54.0	52.8	46.8	53.4
	4	0.0	4.6	12.0	18.6	33.0	29.4	36.6
	5	0.0	0.0	15.6	31.8	28.2	33.6	33.8
	6	0.0	0.0	11.8	18.0	27.0	27.6	27.6
	Mean blood level	2.1	9.8	25.9	36.1	39.0	43.7	37.3
	SD	±3.25	±11.1	±16.8	±17.15	±11.14	±16.12	±11.53
P-5 (29.96 μ)	1	9.6	5.4	10.8	15.6	31.8	46.5	12.0
	2	3.0	6.0	11.4	27.0	16.2	33.0	24.6
	3	0.0	2.4	11.4	18.0	30.6	27.6	37.2
	4	0.0	4.6	11.6	17.6	26.4	27.2	35.1
	5	0.0	0.0	4.8	7.2	13.2	12.0	11.4
	6	0.0	0.0	9.0	8.4	16.8	22.2	22.8
	Mean blood level	2.1	3.1	9.8	15.6	22.5	28.1	23.8
	SD	±3.86	±2.67	±2.65	±7.24	±8.07	±11.45	±10.95
P-6 (17.16 μ)	1	3.0	9.6	18.0	13.2	33.6	40.8	47.4
	2	3.0	7.4	9.6	18.0	42.3	54.6	37.2
	3	0.0	4.2	9.6	11.6	16.2	15.6	22.5
	4	0.0	6.8	16.2	21.6	31.2	33.6	43.2
	Mean blood level	1.5	7.0	13.3	16.1	30.8	36.1	37.6
	SD	±1.73	±2.24	±4.39	±4.56	±10.86	±16.24	±10.90
P-7 (10.68 μ)	1	4.8	18.6	35.4	39.0	49.2	45.0	51.6
	2	6.0	31.2	55.2	46.8	43.2	40.2	46.5
	3	0.0	3.6	16.8	15.0	30.6	31.2	34.2
	4	0.0	12.0	35.1	37.5	37.2	45.6	41.1
	5	0.0	18.0	39.0	43.2	45.0	49.2	44.6
	6	0.0	0.0	14.4	16.2	28.8	31.8	28.2
	Mean blood level	1.8	13.9	32.6	32.9	39.0	40.5	41.0
	SD	±2.81	±11.36	±15.13	±13.82	±8.19	±7.54	±8.55

Sample	Trial	Hours: 0.25	0.5	1	3	6	9	24	30
		Blood Levels, mcg./ml.							
Intramuscular solution (control)	1	—	39.6	50.5	70.8	61.2	63.0	—	—
	2	—	32.4	47.4	39.6	37.8	46.8	—	—
	3	—	31.2	42.0	64.8	62.9	61.2	—	—
	4	—	37.0	45.7	66.0	63.7	68.3	—	—
	5	—	37.2	46.3	60.0	60.6	61.2	58.2	52.8
	6	—	21.0	37.8	45.6	51.0	49.8	45.6	59.3
	7	—	24.0	45.6	63.0	57.6	60.0	52.2	71.4
	Mean	—	31.8	45.0	58.5	55.7	57.7	52.0	50.2
	SD	—	±7.01	±4.07	±11.50	±9.29	±9.29	±6.31	±16.4
Intravenous solution (control)	1	67.2	63.5	61.2	61.0	62.3	55.4	—	—
	2	68.0	87.0	90.0	63.6	69.0	64.1	—	—
	3	64.0	59.4	55.8	56.3	50.4	61.2	49.2	53.4
	4	49.8	48.0	47.4	49.8	39.5	37.2	39.6	38.3
	5	55.7	50.2	28.8	55.7	49.8	58.2	53.3	50.2
	Mean	60.9	61.6	56.6	55.5	54.2	55.2	47.4	47.3
	SD	±7.75	±15.36	±22.32	±8.66	±11.66	±10.58	±7.14	±7.94

[a] Dose 500 mg. suspended with the aid of Marasperse CB (1% of solid weight).

Table II—Comparison of Relative Areas under the Mean Blood Level Curves between 0 and 30 hr. and Percent of Intravenous Dose after Administration of 500 mg. of Phenobarbital Acid or Its Equivalent of the Sodium Salt to Beagle Dogs[a]

	Intravenous Solution	Intramuscular Solution	Sample Description: Phenobarbital Suspensions Injected Intramuscularly — Sample P-4 (Mean Diameter, 6.63 μ)	Sample P-5 (Mean Diameter, 29.96 μ)	Sample P-6 (Mean Diameter, 17.16 μ)	Sample P-7 (Mean Diameter, 10.68 μ)
Relative areas under mean curve	1563.2(5)	1587.6(6)	983.0(6)	507.2(6)	649.8(4)	952.8(6)
Percent of intravenous dose	100	101	62.9	32.4	41.5	60.9

[a] Numbers in parentheses indicate the number of dogs used to make the blood level curve.

something less than the absolute surface area of the injected mass. The apparent surface area of the injected mass is not known.

The possibility of controlling blood levels of phenobarbital for an extended period of time by regulation of particle size and size distribution of a single intramuscular dose is evident from these data. There was much variability in the individual blood level data obtained with each particle suspension during the first 3–6 hr., but it was not attributed to the dissolution and release pattern from the injected dose. This variability was probably due to a lower concentration of the drug in the body of the dog and its rapid distribution to the various tissues, leaving very little drug in the blood at the time of sampling. Based on the mean intravenous blood level estimate, the "apparent volume of distribution" was approximately 8.8 l.; since the blood volume in dogs weighing between 10 and 15 kg. is about 7% (700–1050 ml.) of body weight, the greater part of the drug must be in other tissues of distribution. As more drug is absorbed from the intramuscular suspensions, the relative standard error becomes smaller.

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Spectrofluorometric Assay of Apomorphine in Brain Tissue

W. KENT VAN TYLE and A. M. BURKMAN

Abstract □ A fluorometric technique has been developed that accurately and reliably measures submicrogram quantities of apomorphine in brain tissue. The assay involves an immiscible solvent extraction procedure which is highly efficient and makes possible the detection of apomorphine in concentrations of 0.1 mcg./g. tissue. The fluorometric assay is about 40 times more sensitive than the spectrophotometric method currently in use.

Keyphrases □ Apomorphine—spectrofluorometric analysis of submicrogram quantities in brain □ Spectrofluorometry—analysis of submicrogram quantities of apomorphine in brain

The increasing interest in apomorphine and related aporphines as centrally active behavioral and emetic stimulants has generated many questions concerning the CNS distribution of these compounds (1, 2). Using a spectrophotometric assay, Kaul *et al.* (3–7) studied apomorphine organ distribution and metabolism but were unable to estimate reliably the CNS levels due to the insufficient sensitivity of their method. Although a variety of techniques have been devised for the compound's determination (8, 9), none provides the sensitivity required to determine submicrogram quantities of apomorphine in small volumes of biological material.

The observation that crystalline apomorphine emits a bluish fluorescence under UV light and the acknowledged fact that fluorometry can be an extremely sensitive assay technique suggested the possibility of using a fluorometric method for apomorphine determination.

This report describes the development of such a procedure for estimating microquantities of apomorphine.

EXPERIMENTAL

Spectral Characteristics and Effect of Solvent—The output of the spectrophotofluorometer[1] was fed simultaneously into a photomultiplier microphotometer and an X-Y recorder[2]. A fused quartz cell (10.5 × 10.5 × 46 mm.) was used for all samples, with instrument slit arrangement number 5.

[1] An Aminco-Bowman spectrophotofluorometer equipped with a 1P21 potted photomultiplier tube assembly was used for all fluorescence measurements.

[2] Spectrophotofluorometer, microphotometer, and X-Y recorder were manufactured by American Instrument Co., Inc., Silver Spring, Maryland.