quired for the animal to regain the wink reflex when the stimulus was applied was recorded in minutes. In most cases six rabbits were used for each comparison although in a few cases five were used and in one case 12 were employed. Each pair of esters was tested on the same rabbit's eyes with the right eye being used for one-half of the tests on the hydroxyquinolizidine esters and the left eye for the other half. Concomitantly, the alternate eye of the rabbits was used for the other ester. At no time were both eyes subjected to anesthesia at the same time.

The duration ratio between the pairs of compounds in each animal was obtained by dividing the duration of the 2-hydroxyquinolizidine ester by that of the 3-(2-methylpiperidino)propanol ester. The mean of these ratios was determined and the 95% confidence limits of the mean were calculated assuming a normal distribution for the mean and a chi-square distribution for the squares of the deviations (17). These results are given in Table III.

Observations.—No irritation of the cornea was observed with either series of esters in the concentrations employed. Both the *p*-isoamyloxybenzoate and the *o*-benzoylbenzoate were erratic in the 2-hydroxyquinolizidine series with respect to the comparative durations. The latter, in particular, exhibited an unpredictable behavior with the duration ratios varying widely (0.83 to 3.86). For this reason, the 95% confidence interval varies more widely than any of the other pairs tested. A further observation of interest was that the 2-hydroxyquinolizidine esters induced much less xerophthalmia than the corresponding 3-(2-methylpiperidino)-propanol esters.

SUMMARY

1. Fifteen new substituted benzoate esters of 2-hydroxyquinolizidine have been synthesized and described because of an interest in their comparative local anesthetic activity with the corresponding esters of 3-(2-methylpiperidino)propanol.

2. Four new methyl esters of substituted benzoic acids have been synthesized and described in the course of the investigation.

3. Several of the newly synthesized esters have been compared with their counterparts obtained from the esterification of 3-(2-methylpiperidino)-propanol for duration of corneal anesthesia in the rabbit. The observed results indicate that an enhanced duration of action can be expected as a general trend when comparing the two esters derived from the same acid. The duration ratios varied from 1.09 (± 0.11) for the *o*-hydroxybenzoates to 2.38 (± 0.36) for the *o*-(*n*-butoxy)benzoates.

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Release of Drug from a Self-Coating Surface

Benzphetamine Pamoate Pellet

By W. I. HIGUCHI† and W. E. HAMLIN

The problem has been examined in which the rate of release in acid solution of an amine drug from a pellet of a weak acid salt of the amine is controlled by a coat which is formed by precipitation of a weak acid onto the pellet surface. A detailed mathematical analysis has been carried out and an expression for the rate of release is presented. The theory has been applied to data on the release of the drug from benzphetamine pamoate pellets.

RECENT STUDIES by Morozowich, *et al.* (1), on the release of benzphetamine from pellets of benzphetamine pamoate in 0.12 *N* hydrochloric acid medium led these investigators to propose that in this instance the rate was controlled by a layer of pamoic acid which was deposited on the pellet surface according to the reaction:

$$\text{benzphetamine pamoate (pellet)} \xrightarrow{H^+} \text{benzphetamine (solution)} + \text{pamoic acid (pellet surface)}.$$

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This suggestion was based upon the reality of the pamoic acid layer determined by assay of the pellets exposed to the solution and the relative small effect of agitation on the rate of benzphetamine release.

It appeared worthwhile to examine this problem further, both experimentally and theoretically, particularly since the basic considerations may apply to other situations.[1] The results of a detailed analysis of this problem are presented in this report.

THEORY

The Model and Assumptions.—We will assume that the above picture is correct, *i.e.*, the rate of benzphetamine release is controlled by diffusional processes in the pamoic acid layer. Our concern will be only for the case in which the dissolving medium is acidic.

The model is illustrated in Fig. 1. At some time after the pellet is placed in the acid solution, a layer of pamoic acid of thickness s has formed on the surface. The H^+ must diffuse through this layer and react with the pamoate ion to precipitate more acid, releasing the benzphetamine cation

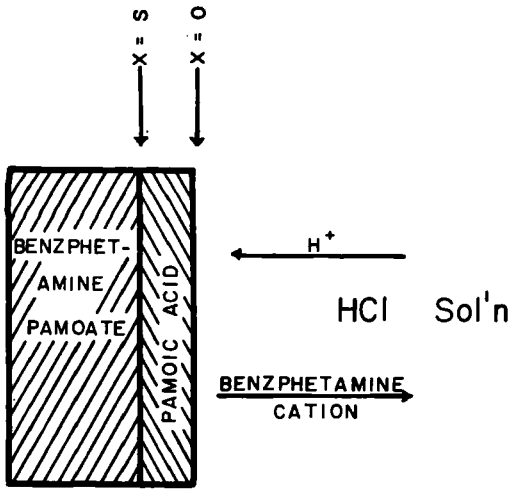

Fig. 1.—Model for the release of benzphetamine from benzphetamine pamoate in hydrochloric acid solution. See text for explanation.

which then diffuses out through the layer. Some of the benzphetamine cation will diffuse out without being released by the H^+ since benzphetamine pamoate has a finite solubility.

In order to make this problem mathematically tractable it is necessary to incorporate the following assumptions into the model: (*a*) quasi-steady-state in the pamoic acid layer; (*b*) Fick's law of diffusion is obeyed, constant effective diffusion coefficients in the layer, and diffusion takes place only in the aqueous phases of the layer; (*c*) effects of liquid diffusion layer negligible; (*d*) initial non-steady-state effects negligible. Some of these assumptions will be examined later.

Concentration-Equilibria-Diffusion Relations.—Let us now obtain an expression for the instantaneous release rate of benzphetamine at a given pamoic acid layer thickness s with the assumption that steady state concentration gradients are present. The appropriate boundary conditions for the problem are $C_{H^+} = C^\circ_{H^+}$, $C_{B^+} = C^\circ_{B^+}$, $C_{P=} = C^\circ_{P=}$, and $C_{H_2P} = K_l$ = constant at $x = 0$ where C_{H^+}, C_{B^+}, $C_{P=}$, and C_{H_2P} are the H^+, benzphetamine-H^+, pamoate anion, and the pamoic acid (in solution) concentrations, respectively. At $x = s$ we have $C_{H^+} = C^s_{H^+}$, $C_{B^+} = C^s_{B^+}$, $C_{P=} = C^s_{P=}$, and $C_{H_2P} = K_l$.

As before (4) we may write down the appropriate differential equations for diffusion and simultaneous chemical reaction in a one dimensional situation. Throughout the region $0 \leq x \leq s$ we have

$$D_{P=}\left(\frac{d^2C_{P=}}{dx^2}\right) + \Phi = 0 \qquad \text{(Eq. 1)}$$

$$D_{H^+}\left(\frac{d^2C_{H^+}}{dx^2}\right) + 2\Phi = 0 \qquad \text{(Eq. 2)}$$

$$D_{B^+}\left(\frac{d^2C_{B^+}}{dx^2}\right) = 0 \qquad \text{(Eq. 3)}$$

A forth equation involving H_2P is omitted because $C_{H_2P} = K_l$ is independent of x as long as solid pamoic acid is present. In Eqs. 1 to 3 the D's are the effective diffusion coefficients for the species indicated and Φ is the rate of reaction per unit volume for the reaction $P^= + 2H^+ = H_2P$ (aq.) = H_2P (s). Since there will be no formation or disappearance of benzphetamine in the pamoic acid layer, the second derivative of C_{B^+} is always zero.

If Eqs. 1 and 2 are combined and integrated over the limit $x = 0$ to $x = s$, we obtain

$$G = \frac{2D_{P=}(C^s_{P=} - C^\circ_{P=}) + D_{H^+}(C^\circ_{H^+} - C^s_{H^+})}{s} \qquad \text{(Eq. 4)}$$

Furthermore, integration of Eq. 3 over $x = 0$ to $x = s$ gives

$$G = \frac{D_{B^+}(C^s_{B^+} - C^\circ_{B^+})}{s} \qquad \text{(Eq. 5)}$$

In these equations, G is the rate of release of benzphetamine per unit area of pellet surface.

In order to eliminate the unknowns, $C^s_{P=}$, $C^s_{H^+}$, and $C^s_{B^+}$ from the expression for G, it is necessary to introduce the appropriate equilibrium relationships. These are the solubility product expressions for benzphetamine pamoate

$$K_{sp} = C^2_{B^+}C_{P=} \qquad \text{(Eq. 6)}$$

and the dissociation constant expression for pamoic acid

$$K_A = \frac{C^2_{H^+}C_{P=}}{C_{H_2P}} = \frac{C^2_{H^+}C_{P=}}{K_l} \qquad \text{(Eq. 7)}$$

If Eqs. 4–7 are combined, we may obtain the following relation upon eliminating G

[1] A similar but simpler situation is that of drug release from emulsion ointment bases (2). Another analogous situation has been reported by Levy and Procknal (3) for the release of acetylsalicylic acid from aluminum acetylsalicylic acid pellets in a basic aqueous medium which involves the formation of a water-insoluble aluminum compound on the surface of the pellets.

$$C^{s}_{B^+} = \frac{D_{B^+} C^{\circ}_{B^+} + 2D_{P=} [(K_{sp}/C^{s2}_{B^+}) - (K_A K_l / C^{\circ 2}_{H^+})] + D_{H^+} C^{\circ}_{H^+}}{D_{B^+} + D_{H^+} (K_A K_l / K_{sp})^{1/2}} \quad \text{(Eq. 8)}$$

Now the numerical values[2] for K_{sp}, K_A, and K_l are $K_{sp} = 3 \times 10^{-10}$, $K_A = 1 \times 10^{-6}$, and $K_l = 4 \times 10^{-6}$. Substitution of these into Eq. 8 gives

$$C^{s}_{B^+} = \frac{D_{B^+} C^{\circ}_{B^+} + 2D_{P=} \{[(3 \times 10^{-10})/(C^{s2}_{B^+})] - [(4 \times 10^{-12})/(C^{\circ 2}_{H^+})]\} + D_{H^+} C^{\circ}_{H^+}}{D_{D^+} + 0.12 D_{H^+}} \quad \text{(Eq. 9)}$$

In the experiments to be discussed later, $C^{\circ}_{H^+} \sim 0.01$ to 0.1 M. For this range of $C^{\circ}_{H^+}$ it can be shown by consideration of Eqs. 4 and 5 that to within a factor of ten, $C^{s}_{B^+} \sim C^{\circ}_{H^+}$. Therefore, the middle terms in the numerator of Eq. 9 are negligible compared to the first and last terms. Hence for our present purposes, Eq. 10 may be written

$$C^{s}_{B^+} = \frac{D_{B^+} C^{\circ}_{B^+} + D_{H^+} C^{\circ}_{H^+}}{D_{B^+} + 0.12\, D_{H^+}} \quad \text{(Eq. 10)}$$

Now if this expression is combined with Eq. 5 we get

$$G = \frac{D_{B^+}}{s} \left(\frac{D_{H^+} C^{\circ}_{H^+} - 0.12\, D_{H^+} C^{\circ}_{B^+}}{D_{B^+} + 0.12\, D_{H^+}} \right) \quad \text{(Eq. 11)}$$

Benzphetamine Release vs. Time Relation.— We may now relate G to the variation in thickness s with time t by means of the following equation

$$G = A \frac{ds}{dt} - {}^1/_2\, V C^{s}_{B^+} \frac{ds}{dt} \quad \text{(Eq. 12)}$$

where A is the concentration of benzphetamine in the solid benzphetamine pamoate and V is the volume fraction of the liquid (aqueous) phase in the pamoic acid layer. The first term on the right side of Eq. 12 is the amount of benzphetamine leaving[3] the solid benzphetamine pamoate per unit time and unit area, and the second term is the amount of benzphetamine needed to re-establish the steady-state concentration gradient in the region $0 \leq x \leq s$ after the change in s.

Letting

$$\Delta C = A - ({}^1/_2)\, V C^{s}_{B^+} \quad \text{(Eq. 13)}$$

combining Eqs. 11 and 12, and separating variables, we get

$$s\,ds = \frac{D_{B^+}}{\Delta C} \left[\frac{D_{H^+} C^{\circ}_{H^+} - 0.12\, D_{H^+} C^{\circ}_{B^+}}{D_{B^+} + 0.12\, D_{H^+}} \right] dt \quad \text{(Eq. 14)}$$

Integrating from $s = 0, t = 0$ to $s = s, t = t$ this becomes

$$s = \left[\frac{2D_{B^+}}{\Delta C} \left(\frac{D_{H^+} C^{\circ}_{H^+} - 0.12 D_{H^+} C^{\circ}_{B^+}}{D_{B^+} + 0.12\, D_{H^+}} \right) t \right]^{1/2} \quad \text{(Eq. 15)}$$

Then for the amount M released per cm.^{2} of surface we have

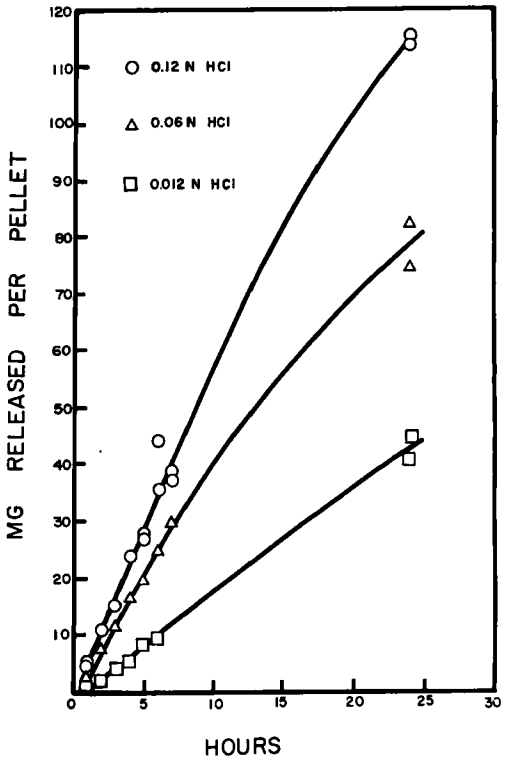

Fig. 2.—Data on the release of benzphetamine from pellet of benzphetamine pamoate in aqueous solutions of hydrochloric acid at 37°.

$$M = S\Delta C = \left[2D_{B^+} \Delta C \left(\frac{D_{H^+} C^{\circ}_{H^+} - 0.12\, D_{H^+} C^{\circ}_{B^+}}{D_{B^+} + 0.12\, D_{H^+}} \right) t \right]^{1/2} \quad \text{(Eq. 16)}$$

In order to make Eq. 16 workable, it is necessary to have estimates of D_{B^+}, D_{H^+}, and ΔC. For the diffusion coefficients we may write

$$D_{B^+} = f D'_{B^+}$$

and (Eq. 17)

$$D_{H^+} = f D'_{H^+}$$

where the D primes are the diffusion coefficients of the ions in pure aqueous phase. Therefore, f represents the effects of volume fraction, particle shapes, and other factors. The value of f may be estimated from approximate theories (6) based on data for other systems. We find that, if $V \sim 0.5$, $f \sim 0.25 \pm$ factor of 50%. The value for D'_{B^+} may be estimated from the Stokes-Einstein law to be about 5×10^{-6} cm.^{2} sec.$^{-1}$ and $D'_{H^+} \sim 3 \times$

[2] These are for 37°. The K_A value was determined at 25° by a potentiometric titration method in various concentrations of aqueous dimethylacetamide solutions and the results were extrapolated to 100% water. The temperature dependence of K_A values in water for carboxylic acids are usually small (5) near room temperatures. Since K_A for salicylic acid is about 1×10^{-3}, our value for pamoic acid is reasonable.

[3] It is assumed that the rate of change in thickness of the pamoic acid layer equals that of the pamoate layer. This is consistent with experimental observations.

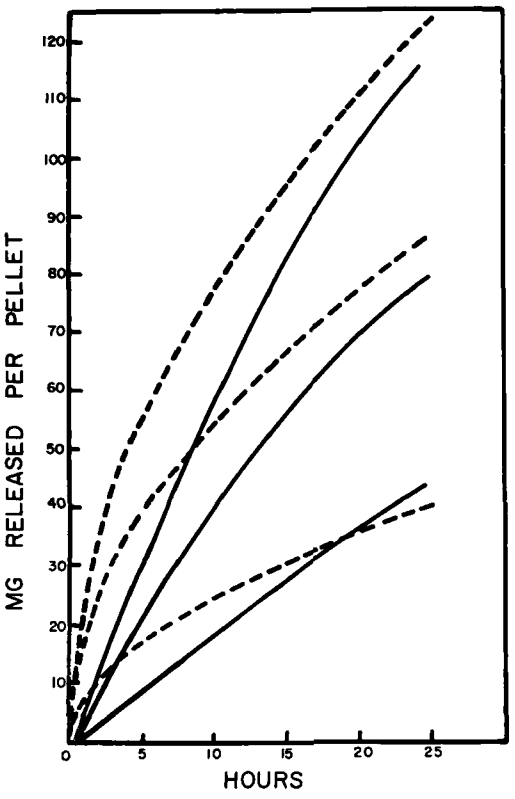

Fig. 3.—Comparison of experimental results (closed curves) with Eq. 19 of theory (dotted curves).

10^{-5} cm.^{2} sec^{-1} from the literature (7). Finally, since it can be easily shown from Eq. 10 and the above estimates of the diffusion coefficients that $C^{\circ}_{D^+} \ll A$ for our experimental conditions, we have $\Delta C \simeq A$. Therefore, $\Delta C \simeq 2.6 \times 10^{-3}$ moles ml.$^{-1}$ since the density of benzphetamine pamoate is 1.12 and the equivalent weight is 433. With these values for D_{B^+}, D_{H^+}, and ΔC, and noting that $C^{\circ}_{H^+} \gg 0.12\ C^{\circ}_{B^+}$ for our experiments, Eq. 16 becomes

$$M \simeq 1.5 \times 10^{-4} (C^{\circ}_{H^+} t)^{1/2} \text{ moles benzphetamine release per cm.}^2 \quad \text{(Eq. 18)}$$

if $C^{\circ}_{H^+}$ is in moles ml.$^{-1}$ and t is in seconds.

EXPERIMENTAL

Because of the large number of parameters in the theory, the values of which are known only approximately, it appeared that an evaluation of the problem on the basis of a single experiment would be difficult. Therefore, it was decided to vary an obviously important factor, the hydrogen ion concentration, to see whether the results of the experiments would fall self-consistently into the framework of the theory.

A series of experiments employing the hanging pellet method (8) at 0.12, 0.06, and 0.012 *N* hydrochloric acid were carried out. The pellets, which were one-half inch diameter and approximately two millimeters thick, were prepared by compressing benzphetamine pamoate in a potassium bromide die. The die was evacuated and a compressional force of 10 tons was applied. The pellets were mounted on aluminum plates by means of wax. Excess wax and any trace of magnesium stearate, which was used to coat the inner surfaces of the die, were removed from the one-half inch diameter surface by careful scrapping with a razor blade. Each pellet was then exposed to 100 ml. of the hydrochloric acid solutions. At a predetermined time a pellet was removed and the amount of drug released by it was determined by U.V. and colorimetric methods. All runs were carried out at 37°.

COMPARISON OF RESULTS WITH THEORY

The data on the release of benzphetamine with time are presented in Fig. 2 along with their best fit curves. Each experimental point represents a different pellet.

These data may be compared with theory in three ways. First, the coefficient, 1.5×10^{-4}, in Eq. 18 may be tested; secondly, the $C^{\circ}_{H^+}$ dependence may be examined; and finally, the time dependence may be tested.

In Fig. 3 the experimental results are compared with the adjusted equation

$$M = 1.25 \times 10^{-4} (C^{\circ}_{H^+} t)^{1/2} \text{ moles cm.}^{-2} \quad \text{(Eq. 19)}$$

which differs from Eq. 18 by only 20% in the coefficient. The surface area of 1.26 cm.^{2} was used with Eq. 19 for the calculation of the theoretical curves.

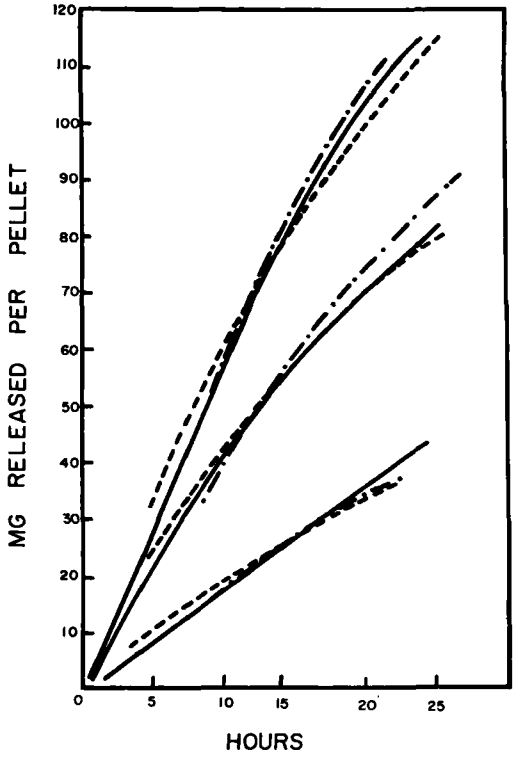

Fig. 4.—Comparison of experimental results (closed curves) with Eq. 20 (–·—·–) and with Eq. 21 (– – – –) of theory.

A close examination of Fig 3 shows that on two of the three tests of the theory with data the theoretical model appears to be in satisfactory agreement with the experiments. Firstly, the experimental $C^{\circ}{}_{H^+}$ dependence correctly fits the square root law[4] at essentially all times. Secondly, within the probable uncertainty of about a factor of two in theory, the coefficient, 1.5×10^{-4}, in Eq. 18, agrees[5] with data at large times for all $C^{\circ}{}_{H^+}$. Thus it appears that the disagreement between experiment and theory exists largely on the third point, the time dependence.

To amplify the idea that it is primarily the time dependence of the model which is at fault, let us consider two modifications of Eq. 18 which give better fit to the data. In Fig. 4 the data are compared with the following two equations

$$M = 1.4 \times 10^{-4} (C^{\circ}{}_{H^+})^{1/2} (t - \tau_1)^{1/2} \quad \text{(Eq. 20)}$$

and

$$M = 1.4 \times 10^{-4} (C^{\circ}{}_{H^+})^{1/2} (t^{1/2} - \tau_2^{1/2}) \quad \text{(Eq. 21)}$$

where we have taken τ_1 = six hours and τ_2 = one hour for the calculations. In these equations the coefficient and $C^{\circ}{}_{H^+}$ dependence have been retained from Eq. 18. Only changes in the time dependence, effective mainly at small t, have been incorporated. The meaning of τ_1, in Eq. 20, is that there is effectively a constant lag time, $\tau_1 \sim$ six hours, before the release process begins according to the theoretical model. The meaning of τ_2 in Eq. 21 is that there is not only a small lag time $\tau_2 \sim$ one hour, but there is effectively a small barrier in series with the pamoic acid barrier itself. This small effective barrier would, of course, be most important at small t values when the pamoic acid layer is thin. Actually, the data in Fig. 2 indicate a small lag time the order of one hour. It is more difficult to account for the barrier in series with the pamoic acid layer. It might be partially accounted for by the liquid diffusion layer and partially by a varying pamoic acid layer structure near the surface, *i.e.*, effectively smaller diffusion coefficients in the layer near the surface.

[4] If the coating phenomenon were absent, a linear law would be expected according to Eq. 11 with constant s.

[5] If the coating phenomenon were absent, rates ten to a hundred times greater would be expected.

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Investigation of *in Vivo* Tracer Techniques in Drug Screening Studies

By BILLY D. RUPE, WILLIAM F. BOUSQUET, and JOHN E. CHRISTIAN

An *in vivo* tracer method for screening drugs which act by altering the normal body metabolism of certain elements is described. These studies involved the detection of natruretic or antinatruretic action of six compounds. Further extensions of the present work are also discussed.

THE RELATIVELY RECENT development of large volume liquid scintillation counters ("Whole Body Counters") (1, 2) has made possible research on the development of new methodology for the qualitative and quantitative evaluation of pharmacologically active substances. *In vivo* assay of gamma ray emitting radioisotopes, as made possible with large volume liquid scintillators, is particularly attractive to the research pharmacologist in drug screening studies. The obvious advantages of this technique are three: (*a*) observations may be made on the intact animal; (*b*) serial observations may be made on the same animal over extended periods of time; and (*c*) it should be possible to reduce the size of experimental groups of animals. This technique has not been exploited to date.

One such counter is the Purdue University Small Animal Counter (PUSAC). The specifications and operating characteristics of this counter are fully described in the literature (2). The PUSAC and similar counters now being commercially produced are of such size that mice, rats, or guinea pigs may be used as experimental animals.

Large volume scintillation counters should

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