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# The use of natural membranes for in vitro determination of absorption rates of drugs from suppository bases

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## Summary

The use of natural membranes in the determination of absorption rates of drugs from suppositories was investigated in vitro using the isolated rectums of albino rabbits as natural membranes. Isolated rectums were cut off from the anaesthetized animals just after they died.

Acetaminophen (I) in 3 different particle sizes was used as the test drug. Three fatty type suppository bases were chosen as the test bases.

Absorption of I from suppositories prepared with cacao butter and Witepsol H15 was also investigated in vivo.

The amount of the test drug that passed through the natural membrane was determined spectrophotometrically. The results were calculated from the percentage of the dose applied. The ratio of the area under the concentration–time curve to the dose given by rectal route in vivo, was named the "per cent apparent amount of the drug absorbed".

The relationship between the time and the amount of the test drug absorbed or passed into the test media was linear in both in vivo and in vitro tests, after a lag time of 40 min. However, it became asymptotic in the in vitro tests,when per cent amount of drug passed through the membrane exceeded the 14% of the total drug present in the suppository. When the slopes of these linear relationships were compared, it was seen that the larger the particle size (within the range used, i.e. 088–250 μm) the higher the rate of transfer in vitro or absorption in vivo from fatty suppository bases.

Depending on the in vivo/in vitro correlation, it was concluded that isolated rectums of rabbits can be successfully used in the investigation for the control of formulation parameters of suppositories.

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## Introduction

Pharmaceutical factors such as solubility, particle size (Polderman et al., 1973), partition coefficient, $pK_a$, concentration of active substances, composition, melting range, viscosity, spreading in situ of suppository bases (Rutten-Kingma et al., 1979) affect the absorption rate and extent of drug absorption from suppositories. Several studies have been done to investigate how those factors affected on absorption, both in vivo (Parrott and Matheson, 1977) and in vitro (de Blaey and Ruten-Kingma, 1977). Human volunteers participated in some in vivo studies, while rabbits (Ulrich and Wiese, 1973), dogs (Lowenthal et al., 1970) and rats (Cramer et al., 1978) were also used in others.

Active substances should be released from the suppository bases to be available for absorption at the absorption site. Although the best interpretation can be given depending on the results of the tests performed on volunteers, the in vitro tests, complying with the natural conditions of rectum as much as possible, have been used for the comparative investigations of various parameters in the preparation and control of suppository formulations (Bhavnagri, 1969; Thomas and McCormack, 1971).

In this study, the use of natural membranes in the comparative determination of absorption rates of drugs from rectal suppositories is reported. Isolated rectums of albino rabbits—the biggest laboratory animal readily available and easy to breed—were used as natural membranes. Acetaminophen was preferred as the test drug because its pharmacological and pharmacokinetic properties had already been investigated both in man and in rabbits (Liedtke et al., 1979; Shibasaki et al., 1971). Particle size was chosen as a parameter since it is a major pharmaceutical factor affecting the absorption rate.

We also performed in vivo tests in rabbits to see if there is an in vivo/in vitro correlation.

## Materials and methods

*Reagents*

All reagents used were of analytical grade. The test drug (acetaminophen) was supplied from Atabay Drug Company, and was identified by IR [1] and assayed by UV [2] spectrophotometry. Its moisture content was determined by drying to constant weight at 105°C. Its particle size distribution was determined by using the sieving technique. Fractions of 250–210 $\mu$m and 177–149 $\mu$m were chosen as two different ranges of particle sizes. Particles ranging from 105 to 088 $\mu$m were obtained by milling [3] and subsequent sieving [3].

[1] IR spectrophotometer—Perkin Elmer Model 237.
[2] UV spectrophotometer—Zeiss PMQ III.
[3] Sieves—standard ASTM, Gause VA, Mesh 60, 70, 89, 90, 100, 140, 170.
[4] Ball mill—Erweka.

Solubility and dissolution profiles of acetaminophen in different particle sizes were determined using an Erweka Dissolution Tester.

Suppository bases used were Witepsol H15, Witepsol W45 and cacao butter. Cacao butter was tested for the compliance with the specifications given in the Turkish Pharmacopoeia, 1974.

*Preparation of suppositories*

Suppositories, each containing 0.250 g acetaminophen, were prepared for each particle size with the 3 suppository bases mentioned above, according to the fusion technique. Suppositories were then wrapped in glossy paper and stored at room temperature. Each batch was analyzed for its acetaminophen content. Melting points of suppositories were determined at the time they were prepared and after storing at room temperature for approximately 14 months. Melting points were not significantly changed during storage.

*In vitro methods*

The in vitro rate of absorption of acetaminophen was studied using isolated rectums of rabbits as dialysis membranes under specified conditions.

*Procedure*: 100 ml, pH 7.4, isotonic buffer solution was placed in a special glass funnel, having an outer jacket heated by a circulating water bath (Fig. 1).

The terminal 10 cm section of the colon of a rabbit was cut off just after the anaesthetized animal was killed. This isolated segment of intestine was put in a beaker containing Tyrode solution and cleaned by passing the same solution through it. Mesentary and fats were cleaned away if any were present. The isolated rectum so obtained was kept refrigerated in Tyrode solution until use. For the test, it was transferred into a beaker containing approximately 50 ml of the pH 7.4 isotonic phosphate buffer solution at room temperature; 0.5 cm from the anal end of the intestine was tied with a piece of cotton thread, and cut off. After weighing, one suppository, two glass beads and 2 ml of the pH 7.4 buffer solution were placed in it, and the other end of the bag was then tied too.

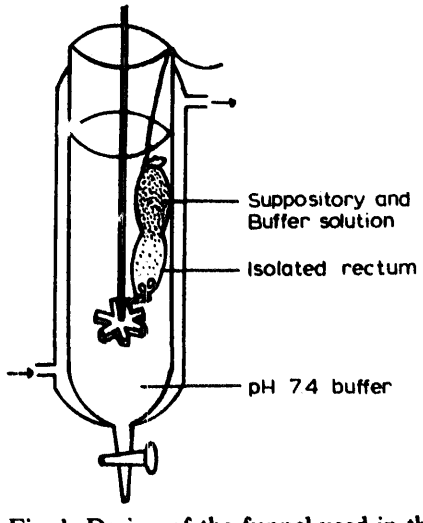


Fig. 1. Design of the funnel used in the in vitro tests.

The dialysis bag of intestine—which remained viable during the test—prepared as described above, was hung into the buffer solution in the funnel. The solution was stirred by a propeller-type stirrer with a stirring rate of approximately 120 rpm. Samples were taken at suitable intervals and the amount of the test drug passed through the natural membrane was determined spectrophotometrically at a wavelength of 243 nm using the pH 7.4 isotonic phosphate buffer as blank. The temperature of the test media was kept at 37 ± 1°C for the cacao butter suppositories and 39 ± 1°C for those of Witepsol W45. Suppositories prepared with Witepsol H15 were examined at both 37 ± 1°C and 39 ± 1°C. The results were expressed as the percentages of the dose applied versus time.

*In vivo method*

The effect of particle size—within the range 2.0/088 μm—on the absorption of acetaminophen was investigated in vivo in rabbits after the rectal administration of the drug in suppositories prepared with the 3 different fatty bases.

*Procedure*

Albino rabbits were fasted for 20 h before the test and no food was given until the end of the test. Three female (weighing 3.1–3.6 kg) and 6 male (weighing 1.9–2.8 kg) rabbits were used. Three or 4 tests for each batch were performed and suppositories were administered to rabbits randomly, but none of the rabbits was re-used for at least one week after the administration of the drug.

A blood sample (3 ml) was taken for the blank and standard curve determination before the application of suppository to rabbit. Blood samples (0.1–0.5 ml depending on the blood concentration of the drug) were taken from ear veins of animals, into tubes containing oxalate anticoagulant mixture, at 20, 40, 60, 90 and 120 min after the administration of the drug. The test was continued for 480 min to determine the pharmacokinetic parameters.

Total acetaminophen concentrations in blood samples were determined by the method of Brodie and Axelrod (1948). It had been previously shown that the glucuronide was the major metabolite for over 90% of the dose administered intravenously to rabbits. Taking this into consideration, the excretion of unchanged acetaminophen was neglected and the two conjugates were considered as a single conjugate (Shibasaki et al., 1971).

## Results and Discussion

The effect of particle size of the drug on the in vitro release of acetaminophen from the 3 different suppository bases, using isolated living natural membrane are shown in Figs. 2, 3 and 4. In all cases, after a lag time of 40 min, the relationship between the per cent amount of drug passed into the test medium and the time was linear for a while, but then the curve started to level off when the amount released reached about 14% of the total acetaminophen content of the suppository used in the test. The concentration of acetaminophen in the test medium,at the time when the curve started to level off, was only 1.74% of the solubility of the drug under the test conditions. In the case of the cacao butter suppositories, the tests were performed at

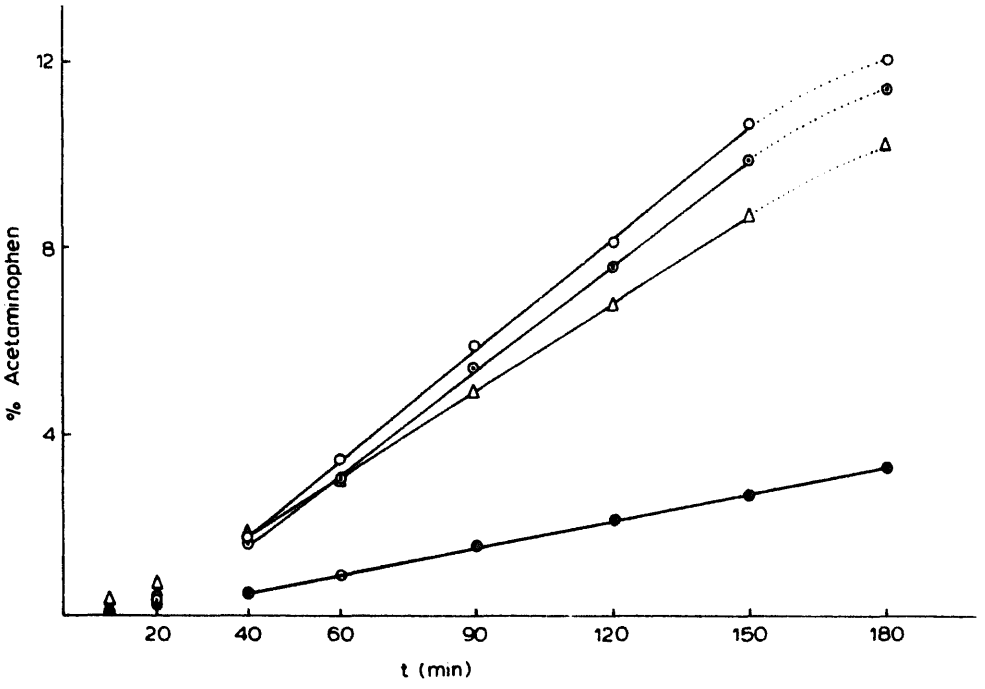


Fig. 2. % acetaminophen passed into the test medium versus time plots at 37 ± 1°C using suppositories prepared with cacao butter. ○, original powder, ⊙, 250/210 μm; △, 177/149 μm; ●, 105/088 μm.

37 ± 1°C and for all particle size distribution ranges studied, the curve did not become asymptotic within the duration of the tests because per cent amounts of drug passed into the test media through the natural membranes did not reach 14% of the dose applied. A similar situation was also observed for Witepsol H15 when the test were performed at 37 ± 1°C. The resolution was very poor at this temperature because of the very slow release of the drug from the suppositories due to the high viscosity of the base. The rate of release and transfer of drug into the test media in vitro increased at 39 ± 1°C since the base became less viscous and the melting time of suppositories was shortened from 40 to 10 min.

The results of the regression analysis of the linear portions of the plots are shown in Tables 1, 2 and 3. Each point is the mean of 3 or 4 tests. It is apparent from the tables that the regression ($r$) and the correlation ($r^2$) coefficients are closer to unity, when they are calculated from the time-averaged per cent amount of drug passed through the membrane, than from those which are calculated from all points.

Neither the weights of rabbits, whose rectums were used in the in vitro tests nor the duration of the storage time of the isolated rectums in Tyrode solution in the refrigerator before the test showed any significant effect on the in vitro absorption rate (per cent amount of drug passed through the membrane).

The slopes of the regression lines of the in vitro results are related to the rate of in vitro absorption. Comparing these slopes, it appears that the larger the particle size within the range tested, i.e., 250/088 μm, the faster the rate of transfer of acetaminophen from fatty-type suppositories through the isolated rectum into the test medium.

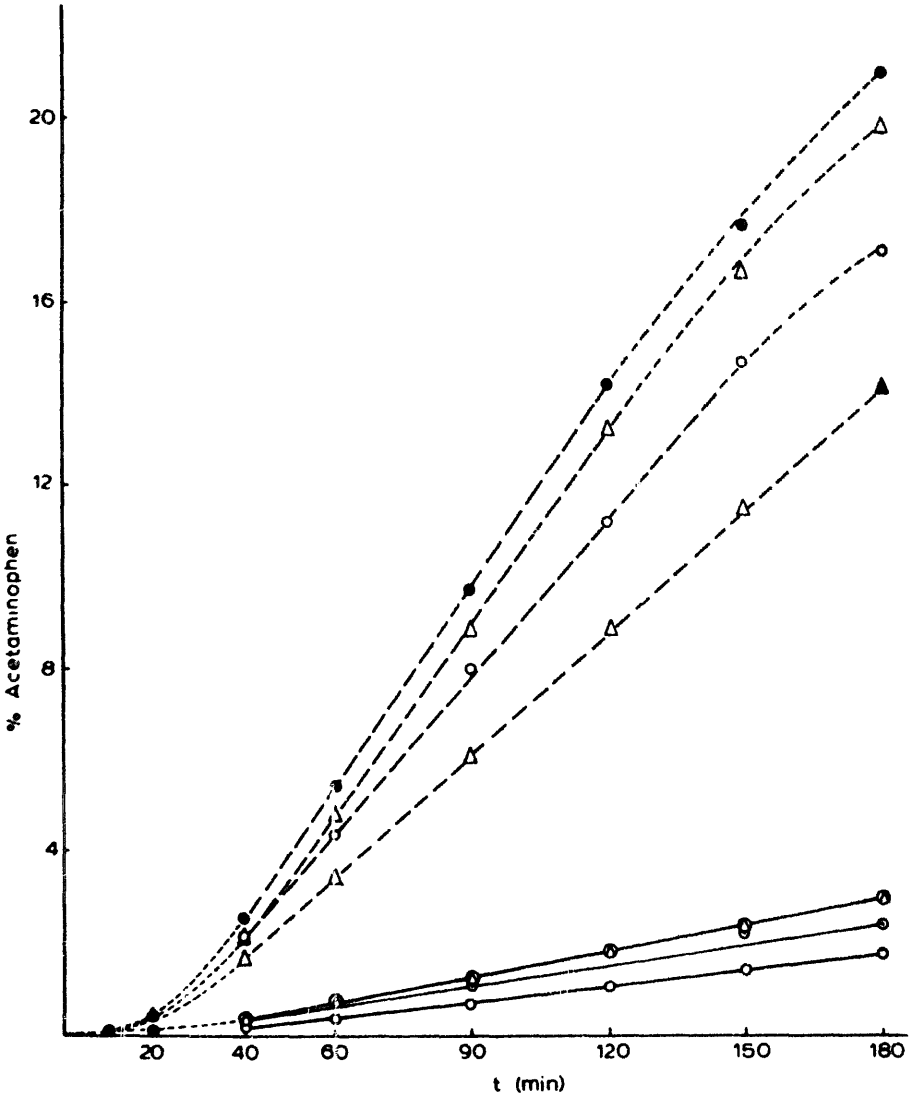


Fig. 3. % acetaminophen passed into the test medium versus time plots at 39 ± 1°C (dotted lines) and at 37 ± 1°C (solid lines) using suppositories prepared with Witepsol H15. ●, 250/210 μm; △, original powder; ○, 177/149 μm; ▲, 105/088 μm.

Application of Hixson–Crowell cube-root law to the results of dissolution tests of acetaminophen in respect of particle size is shown in Fig. 5 (Hixson and Crowell, 1931). The dissolution rate of acetaminophen increases when the particle size increase However, the contrary results, obtained in this study, may possibly be explained by the effect of the static electricity on the surface of small particles, which causes them to agglomerate and decreases the specific surface of the dissolving powder.

Although we could not find a direct correlation between the dissolution rate constants and the rate constants of the in vitro absorption, it appears that there is a rank order correlation between those considering the particle size as shown in Fig. 6.

We performed in vivo tests in rabbits to investigate whether there is an in vivo/in vitro correlation under the stated experimental conditions. Blood concentration–time

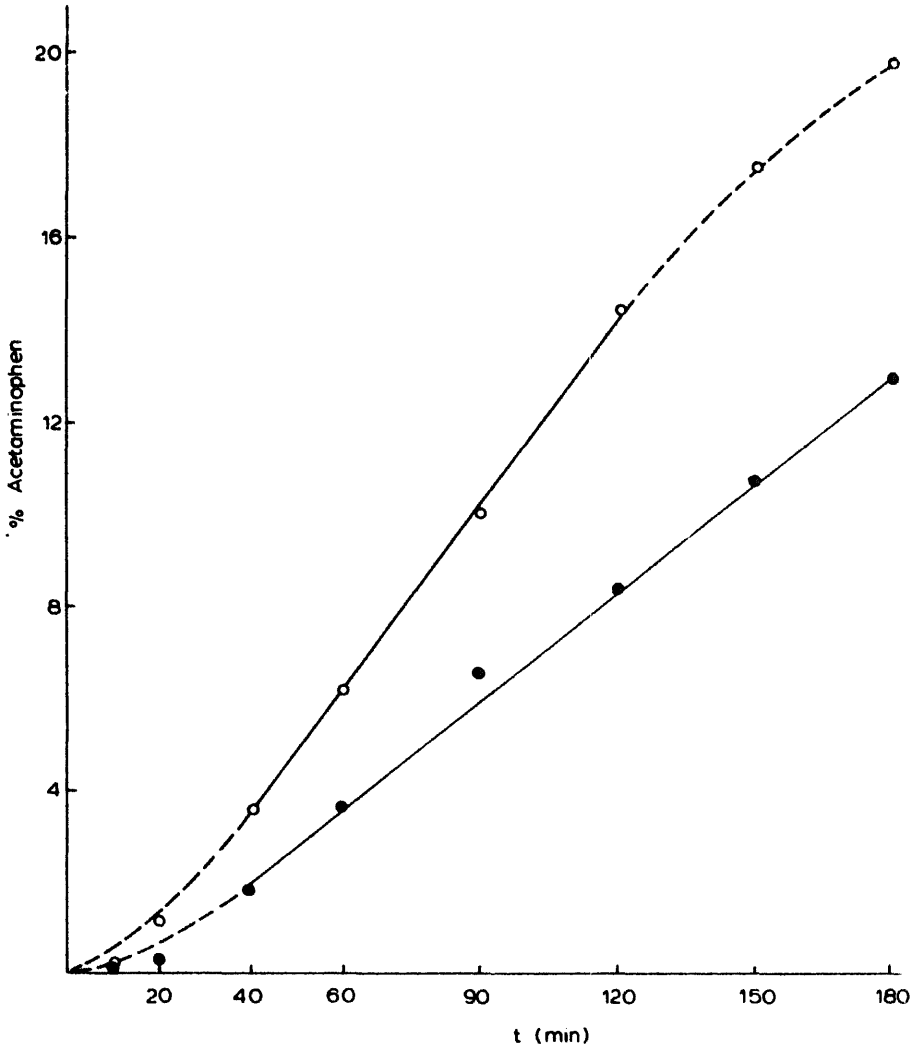


Fig. 4. % acetaminophen passed into the test medium versus time plots at 39 ± 1°C using suppositories prepared with Witepsol W45. ○, original powder; ●, 105/088 μm.

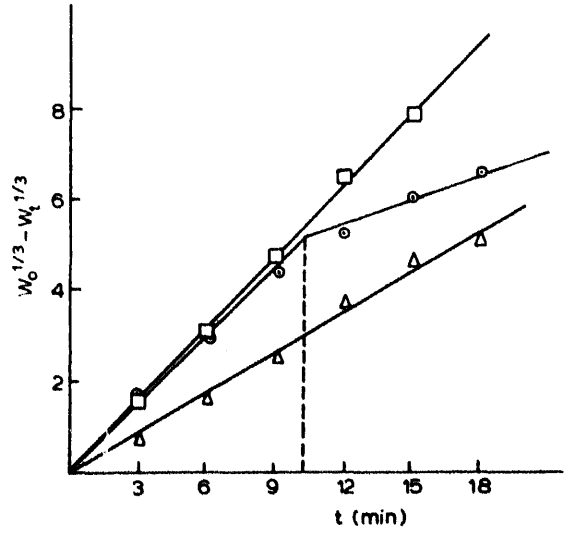


Fig. 5. Application of Hixson–Crowell cube-root law to the results of the dissolution tests of acetaminophen with respect of particle sizes. □, 250/210 μm; ⊙, original powder; △, 105/088 μm.

TABLE 1

STATISTICAL EVALUATION OF RESULTS OF IN VITRO TESTS PERFORMED AT 37 ± 1°C USING SUPPOSITORIES PREPARED WITH CACAO BUTTER

| Particle size | | 250/210 μm | Original powder ($\bar{x}$ = 197.7 μm) | 177/149 μm | 105/088 μm |
|---|---|---|---|---|---|
| Regression line | | y = 0.0723t − 1.1947 | y = 0.0809t − 1.4377 | y = 0.0612t − 0.5719 | y = 0.0192t − 0.2255 |
| $r$ | From $\bar{y}_i$s | 0.999 | 1.00 | 0.996 | 0.982 |
| | From $y_i$s | 0.959 | 0.965 | 0.847 | 0.959 |
| $r^2$ | From $\bar{y}_i$s | 0.997 | 1.00 | 0.993 | 0.964 |
| | From $y_i$s | 0.919 | 0.932 | 0.717 | 0.919 |
| $S_m$ | | 0.0054 | 0.0055 | 0.0063 | 0.00038 |
| $S_n$ | | 0.6378 | 0.6400 | 0.7449 | 0.1415 |
| C.L. ($P<0.05$) | | 0.0837–0.0609 | 0.0925–0.0693 | 0.0746–0.0477 | 0.0200–0.0184 |

For Tables 1, 2 and 3: r, regression coefficient; $r^2$, correlation coefficient; $S_m$, standard error of slopes; $S_n$, standard error of intercept; C.L., confidence limits.

TABLE 2

STATISTICAL EVALUATION OF RESULTS OF IN VITRO TESTS PERFORMED AT 37±1°C AND 39±1°C USING SUPPOSITORIES PREPARED WITH WITEPSOL H15

| Particle size (μM) | | 250/210 | | Original powder | | 177/149 | | 105/088 | |
|---|---|---|---|---|---|---|---|---|---|
| | | 37±1°C | 39±1°C | 37±1°C | 39±1°C | 37±1°C | 39±1°C | 37±1°C | 39±1°C |
| Regression line | Slope | +0.0189 | +0.147 | +0.0112 | +0.139 | +0.0193 | +0.113 | – | +0.0895 |
| | Intercept | −0.0425 | −3.389 | −0.2885 | −3.534 | −0.5745 | −2.359 | – | −1.8789 |
| $r$ | From $\bar{y}_i$s | 0.985 | 1.00 | 0.999 | 0.999 | 0.996 | 1.00 | – | 1.00 |
| | From $y_i$s | 0.615 | 0.980 | 0.882 | 0.990 | 0.756 | 0.940 | – | 0.940 |
| $r^2$ | From $\bar{y}_i$s | 0.970 | 1.00 | 0.998 | 0.997 | 0.992 | 1.00 | – | 1.00 |
| | From $y_i$s | 0.378 | 0.960 | 0.777 | 0.979 | 0.572 | 0.884 | – | 0.884 |
| $S_m$ | | 0.0052 | 0.0095 | 0.0013 | 0.0064 | 0.0042 | 0.0108 | – | 0.0082 |
| $S_n$ | | 0.6065 | 0.7902 | 0.1515 | 0.5366 | 0.4884 | 1.0865 | – | 0.9622 |
| Confidence limits ($P<0.05$) | | 0.0081–0.0297 | 0.126–0.168 | 0.0086–0.0138 | 0.125–0.154 | 0.0104–0.0282 | 0.0896–0.1364 | – | 0.0711–0.1056 |

TABLE 3

STATISTICAL EVALUATION OF THE RESULTS OBTAINED FROM IN VITRO TESTS PERFORMED AT 39 ± 1°C USING SUPPOSITORIES PREPARED WITH WITEPSOL W45

| Particle size | | Original powder | 105/088 μm |
|---|---|---|---|
| Regression line | | $y=0.136t-1.923$ | $y=0.0796t-1.0675$ |
| $r$ | From $\bar{y}_i$s | 1.00 | 0.998 |
| | From $y_i$s | 0.955 | 0.942 |
| $r^2$ | From $\bar{y}_i$s | 1.00 | 0.996 |
| | From $y_i$s | 0.912 | 0.888 |
| $S_m$ | | 0.0133 | 0.0071 |
| $S_n$ | | 1.110 | 0.829 |
| Confidence limits ($P<0.05$) | | 0.166–0.106 | 0.0946–0.0646 |

curves are shown in Figs. 7 and 8 which are the averages of 3 and 4 experiments for each particle size in the case of cacao butter and Witepsol H15, respectively.

The areas under the blood concentration versus time curves were calculated and their ratios to the doses applied were called "apparent per cent of dose absorbed".

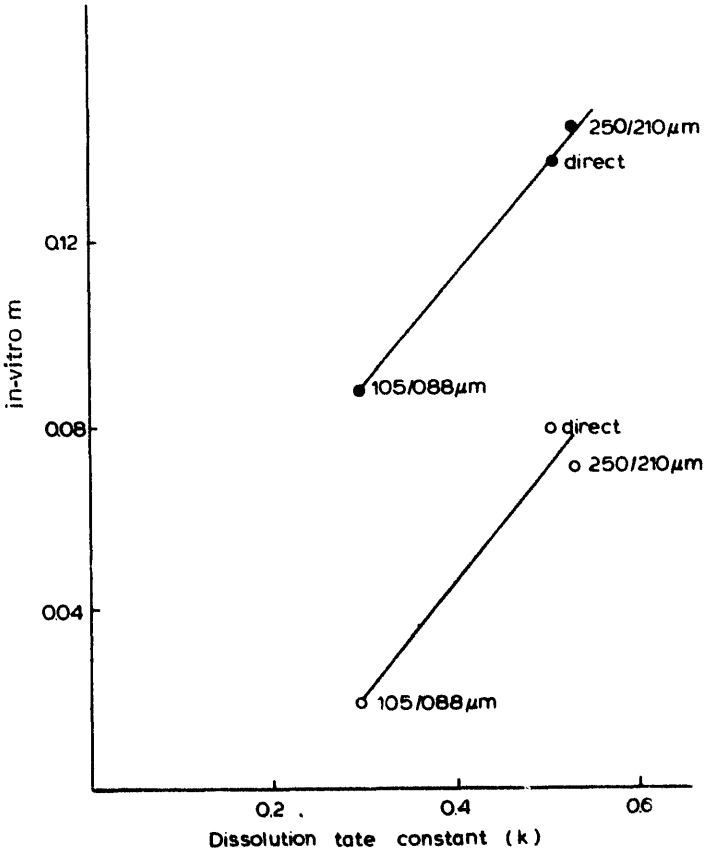


Fig. 6. Correlation between dissolution rate constants and the slopes of the regression lines of the in vitro tests (regression coefficient, $r=0.981$ for Witepsol H15, 0.979 for cacao butter suppositories, $P>0.1$).

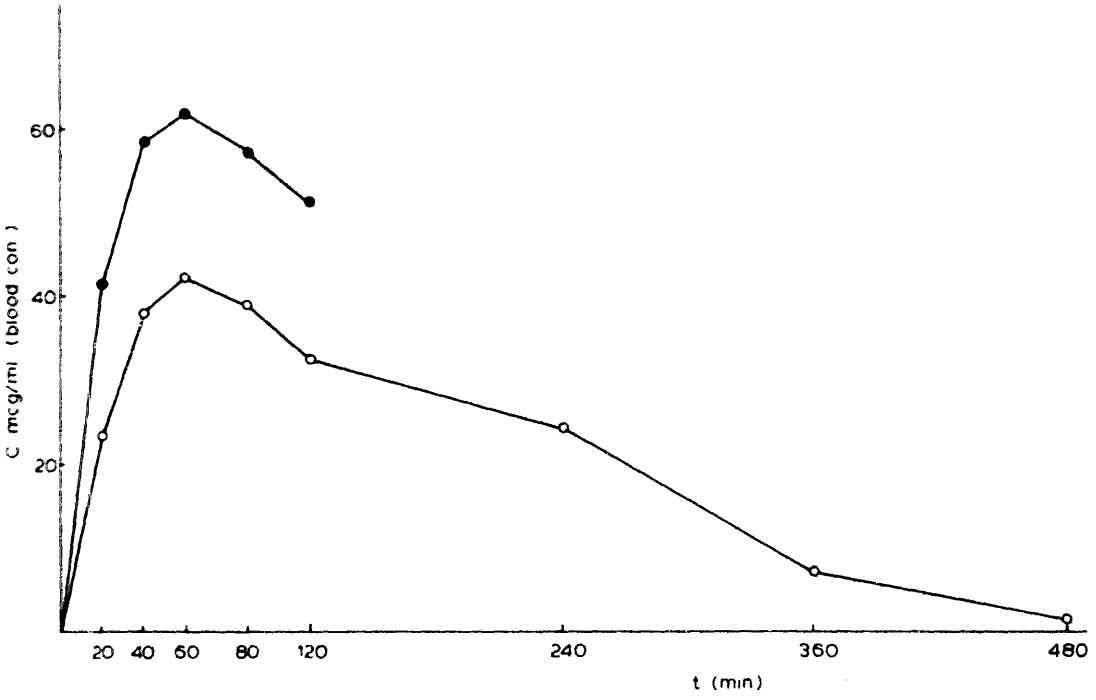


Fig. 7. Blood concentration versus time curves after rectal administration of acetaminophen in two different particle sizes using cacao butter as suppository base. ●, 177/149 μm; ○, 105/088 μm.

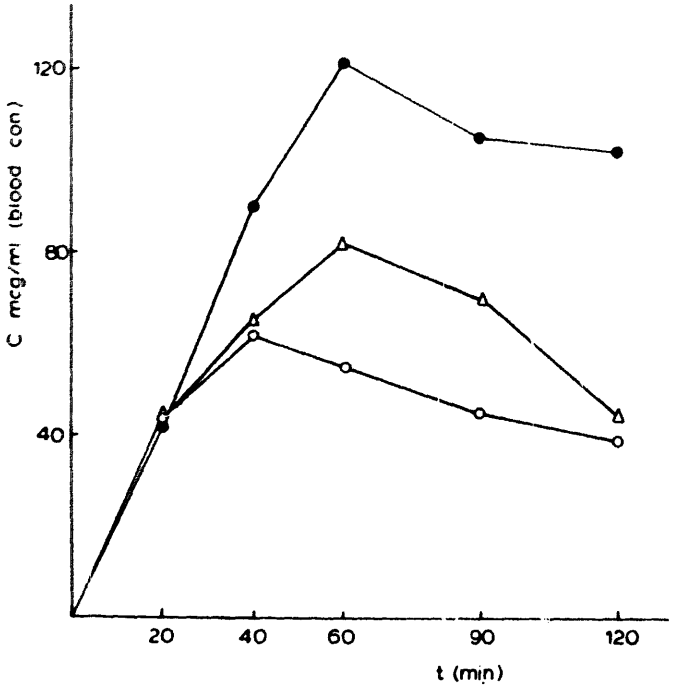


Fig. 8. Blood concentration versus time curves after rectal administration of acetaminophen in 3 different particle sizes using Witepsol H15 as suppository base. ●, 250/210 μm; △, 177/149 μm; ○, 105/088 μm.

We could not calculate the absolute amount since we had not followed the blood concentrations long enough to enable us to calculate the volumes of distribution.

Apparent per cent of drug absorbed versus time plots are shown in Figs. 9 and 10. After a lag time of 40 min, the plots are linear until the end of the test, i.e. 120 min. Observing Figs. 9 and 10, it can be seen that the larger the particle size the faster the absorption rate in vivo. Regression analysis of the linear parts of the results are illustrated in Table 4. The slopes of the lines calculated showed some relationship

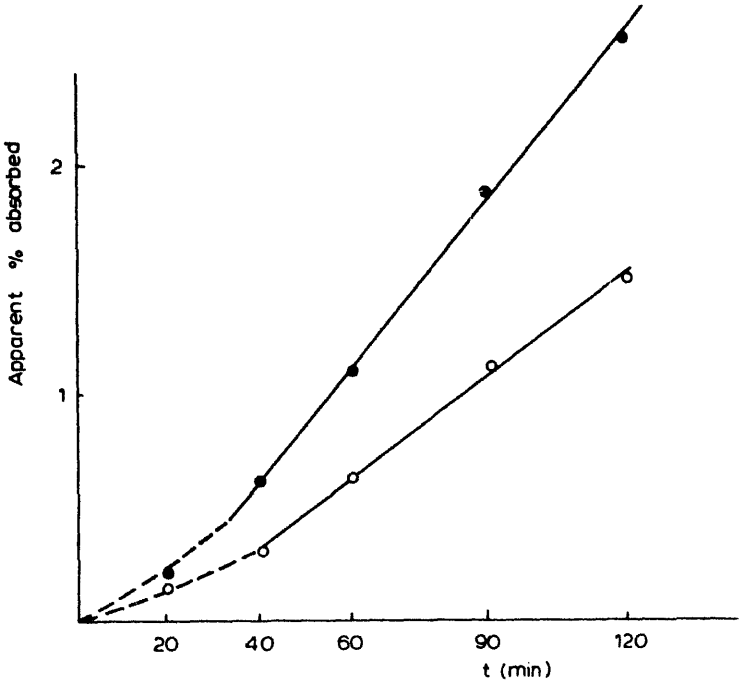


Fig. 9. Apparent % drug absorbed versus time plots after the administration of acetaminophen rectally using cacao butter as suppository base. ●, 177/149 μm; ○, 105/088 μm.

TABLE 4

RESULTS OF THE REGRESSION ANALYSIS OF IN VIVO TESTS PERFORMED USING CACAO BUTTER AND WITEPSOL H15, RESPECTIVELY, AS SUPPOSITORY BASES

| Particle size | | 250/210 μm | 177/149 μm | 105/088 μm |
|---|---|---|---|---|
| $y=mt+n$ | Cacao butter | | 0.0251t−0.3813 | 0.0152t−0.2675 |
| | Witepsol H15 | 0.0445t−1.066 | 0.0279t−0.4560 | 0.0170t−0.0396 |
| $S^2$ | Cacao butter | | 0.0008 | 0.0005 |
| | Witepsol H15 | 0.0011 | 0.0074 | 0.0038 |
| $r$ | Cacao butter | | 1.00 | 0.999 |
| | Witepsol H15 | 1.00 | 0.996 | 0.996 |
| $S_m$ | Cacao butter | | 0.0005 | 0.0004 |
| | Witepsol H15 | 0.0005 | 0.0014 | 0.0010 |
| C.L. ($P<0.05$) | Cacao butter | | 0.0273–0.0229 | 0.0168–0.0136 |
| | Witepsol H15 | 0.0467–0.0423 | 0.0339–0.0217 | 0.0214–0.0126 |

Variance, $S^2$; regression coefficient, $r$; standard error of slopes, $S_m$; confidence limits, C.L.

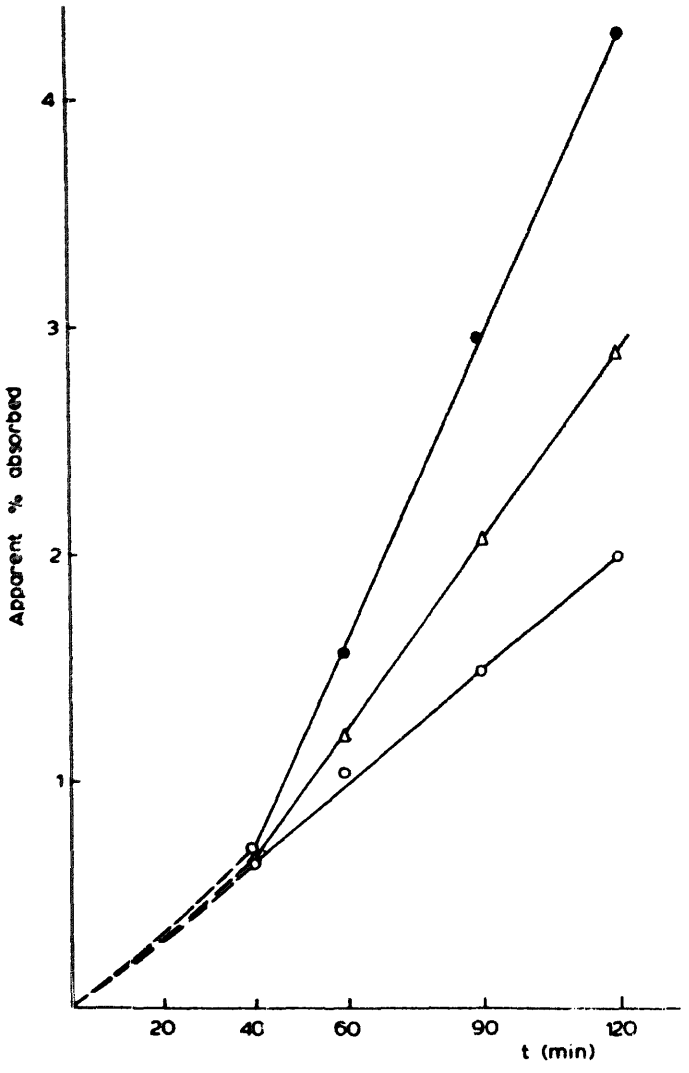


Fig. 10. Apparent % of drug absorbed versus time plots after the administration of acetaminophen rectally using Witepsol H15 as suppository base. ●, 250/210 μm; △, 177/149 μm; ○, 105/088 μm.

with the absorption rate. Taking the particle sizes of acetaminophen into consideration, a quantitative correlation was found between the slopes of in vivo and in vitro results (Fig. 11).

Applying the suppositories prepared with cacao butter and acetaminophen (particle size 105/088 μm) rectally, using a two-compartment open model, some pharmacokinetic parameters in rabbits were calculated and are shown in Table 5. The average half-life of acetaminophen obtained in rabbits was in compliance with the half-life reported in literature after the i.v. administration of the drug to rabbits (Shibasaki et al., 1971).

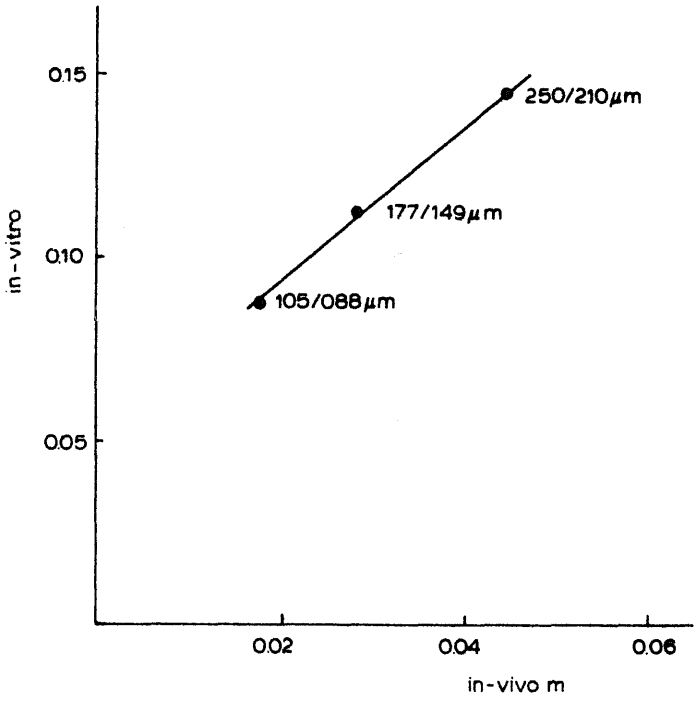


Fig. 11. Correlation between the in vivo and in vitro slopes of regression lines, taking the particle size into consideration. (Regression coefficient of this correlation, $r = 1.00$ $P < 0.01$; variance, $S^2 = 2.08 \times 10^{-6}$.)

TABLE 5

SOME PHARMACOKINETIC PARAMETERS IN RABBITS CALCULATED AFTER THE RECTAL ADMINISTRATION OF ACETAMINOPHEN (105/088 μm) USING CACAO BUTTER AS SUPPOSITORY BASE

| Pharmacokinetic parameters | Assay 1 | Assay 2 | Assay 3 |
|---|---|---|---|
| $\alpha$ | 0.690 $h^{-1}$ | 7.019 $h^{-1}$ | 0.888 $h^{-1}$ |
| $\beta$ | 0.590 $h^{-1}$ | 0.899 $h^{-1}$ | 0.728 $h^{-1}$ |
| A | 9.999 | 7.149 | 6.134 |
| B | 1.904 | 4.951 | 6.367 |
| $k_{21}$ | 0.606 $h^{-1}$ | 3.403 $h^{-1}$ | 0.810 $h^{-1}$ |
| $k_{12}$ | 0.012 $h^{-1}$ | 0.156 $h^{-1}$ | 0.006 $h^{-1}$ |
| $k_2$ | 0.600 $h^{-1}$ | 1.854 $h^{-1}$ | 0.796 $h^{-1}$ |
| $t_{1/2}$ | 1.308 h | 0.772 h | 0.952 h |
| $\bar{t}_{1/2}$ | 1.01 h | standard error, $s = \pm 0.27$ h | |
| | | standard error of average, $s_{\bar{x}} = \pm 0.16$ h | |

$\alpha$, distribution rate constant for two-compartment open model; $\beta$, disposition rate constant for two-compartment open model; A and B, coefficients of the equation describing blood concentration; $t_{1/2}$, half-life; $k_{21}$, $k_{12}$, $k_2$, rate constants of the two-compartment open model.

## Conclusion

From the in vitro/in vivo correlation, it is concluded that isolated rectums of albino rabbits can be successfully used as dialysis membranes in the investigation and control of formulation parameters of suppositories in respect to the absorption characteristics.

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