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Guinea Pig Ear as a New Model for *In Vivo* Percutaneous Absorption

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Abstract □ A new animal model for *in vivo* percutaneous absorption utilizing the hairless, relatively thick skin of the guinea pig ear is proposed. Topical absorption studies were carried out with [^{14}C]hydrocortisone and [^{14}C]testosterone. Systemic studies were also conducted to correct for incomplete urinary excretion. In addition, a single stratum corneum correction factor was developed from published data to enable the guinea pig ear skin to be directly compared with human forearm skin. A comparison of human percutaneous absorption with the corrected guinea pig ear absorption shows a high correlation for both hydrocortisone and testosterone. The effects of ambient changes in relative humidity are also discussed with respect to *in vivo* percutaneous absorption.

Keyphrases □ Guinea pig ear—model for *in vivo* percutaneous absorption, hydrocortisone, testosterone □ Absorption—percutaneous, guinea pig ear, *in vivo* model, hydrocortisone, testosterone □ Hydrocortisone—guinea pig ear, model for *in vivo* percutaneous absorption, testosterone □ Testosterone—guinea pig ear, model for *in vivo* percutaneous absorption, hydrocortisone

Various animal species have been used to study percutaneous absorption *in vivo*. Percutaneous absorption rates of common laboratory rodents such as rats and rabbits have been studied (1). These studies have shown that the back skin of these animals absorb a selected number of substances much more rapidly than the ventral surface of the human forearm (2, 3). Higher species of mammals including hairless dogs (4), rhesus monkeys (5), and miniature swine (1) have also been studied. Of these species, it has been concluded (6) that the rhesus monkey and the miniature swine percutaneous absorption characteristics correlated best with human penetration. The obvious disadvantage of using swine and monkeys include cost and the need for special animal facilities.

One important determinant of percutaneous absorption is the thickness of the stratum corneum. If two areas of skin are sufficiently similar, then one would expect percutaneous absorption to be inversely proportional to the number of stratum corneum layers in the skin. The percutaneous absorption of hydrocortisone from a number of different anatomical sites in humans has been determined previously (7). The number of stratum corneum layers at these sites has also been determined (8). If a proportional relationship between thickness and absorption can be established in humans, this provides a rationale to correct animal model data for thickness. However, the problem with most animal models is that skin with fur generally has a thin stratum corneum (9). Consequently, changes of one or two layers could cause corrections as much as 50% if the stratum corneum is very thin. An ideal animal surface would have to be relatively large and essentially hairless. The guinea pig ear appeared to meet the desired criteria.

EXPERIMENTAL

Male albino guinea pigs, 250–300 g, were individually acclimated for 1 day in metabolism cages[1]. Water and guinea pig chow were given *ad libitum*. For topical application of [^{14}C]hydrocortisone (I) or [^{14}C]testosterone (II), the animals were lightly anesthetized in a bell jar with methoxyflurane[2]. One ear of an animal was sandwiched with medium pressure between two halves of Karush-type dialysis chambers without caps[3]. A 4 μg/cm^3 volume of one of the ^{14}C-labeled compounds reconstituted in acetone was applied in a 40-μl volume to a 1.77-cm^2 area with a flat tip 50-μl syringe[4] to the dorsal surface of the ear. After the acetone had evaporated, the dialysis chambers were removed, and the animals were returned to their metabolism cages. For systemic administration, the ^{14}C-compounds were solubilized first in 50 μl of ethanol and then in 1 ml of sterile saline. The animals were then dosed intraperitoneally with a 0.5-ml volume.

Urine samples were collected daily. The urine was acidified with glacial acetic acid to a pH of 5 to solubilize the magnesium and calcium phosphate precipitates. A 1-ml aliquot was counted on a liquid scintillation counter[5] using 10 ml of liquid scintillation cocktail[6]. The internal standard method using [^{14}C]toluene[7] was used to correct for quench. Relative humidity readings were taken daily using a wet–dry thermometer.

RESULTS

Thickness of the Stratum Corneum and Absorption—Table I presents the determination of:

$$N \times A = K \qquad \text{(Eq. 1)}$$

where N is the number of cell layers in the stratum corneum (8), A is the percent of drug absorbed in 5 days (7), and K is the inverse proportionality constant. These results from human data show that K (forearm) differs from K (back) by only 9.8%. It would be desirable to have more data to confirm this result for different compounds besides hydrocortisone in humans. Nevertheless, one form of verification would be to use a single correction factor to relate guinea pig ear data to the human forearm for a number of different compounds. If Eq. 1 holds, then this

[1] Nalge Co., Rochester, N.Y.
[2] Abbott Lab., North Chicago, Ill.
[3] Belco Glass Co., Vineland, N.J.
[4] Hamilton.
[5] Model 3385 Tri-Carb.
[6] Fisher Scientific Co., Fair Lawn, N.J.
[7] New England Nuclear, Boston, Mass.

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Table I—Inverse Relationship for Hydrocortisone Between Absorption and Stratum Corneum Thickness (Cell Layers)

	Number of Cell Layers[a] (N)	Absorbed/ 5 Days[b], % (A)	Inverse Constant[c] (K)
Forearm (ventral)	21.9	1.04	22.78
Back	16.3	1.26	20.54

[a] See ref. 8. [b] See ref. 7. [c] $K = NA$.

Table II—Recovery of Radioactivity in Urine Following Intraperitoneal (ip) and Topical (t) Administration

Penetrant	n	Admin. Dose/Day (d), % d 1	d 2	d 3	d 4	d 5	Total Absorption Dose, %	Range
I,ip	2	82.1	4.9	1.3	0.9	1.3	93.1	(89.1, 96.8)
I,t[a]	4	0.3	0.8	1.0	0.9	0.9	3.9	(2.8, 5.5)
II,ip	2	62.9	7.2	2.1	1.3	1.0	74.5	(74.4, 74.6)
II,t[a]	3	7.5	7.3	6.4	1.8	2.5	25.9	(22.8, 29.3)

[a] Corrected for recovery following intraperitoneal administration and for stratum corneum thickness of the guinea pig ear.

correction factor would be determined from the relation:

$$A_h = (N_g/N_h)\, A_g \qquad \text{(Eq. 2)}$$

where the subscripts h and g refer to the human forearm and the guinea pig ear, respectively. The values, N_g is 11 and N_h is 21.9, have been determined previously (8, 10). The correction factor, N_g/N_h, is therefore 0.502.

Guinea Pig Ear Absorption—Table II shows the recovery of ^{14}C-labeled compound following intraperitoneal and topical administration of hydrocortisone and testosterone for a 5-day period. The intraperitoneal data were used to correct the topical studies for incomplete excretion. Equation 2 was also used to correct the topical studies for stratum corneum thickness. Figure 1 shows a comparison of the corrected percent of topical hydrocortisone not absorbed for humans (2) and for the guinea pig ear. Similarly, Fig. 2 shows a comparison for testosterone between humans (2), rats (1), rabbits (1), and the guinea pig ear. In Fig. 3, the corrected percent of the dose absorbed is shown for hydrocortisone and testosterone on days 1 and 5. On day 1, the ratio of guinea pig ear to human forearm absorption is nearly 1, whereas by day 5, this ratio is nearly 2.

Effect of Ambient Relative Humidity—In Fig. 4, the corrected percent of drug absorbed by the guinea pig ear is shown for hydrocortisone and testosterone over a 10-day period. Each point is plotted

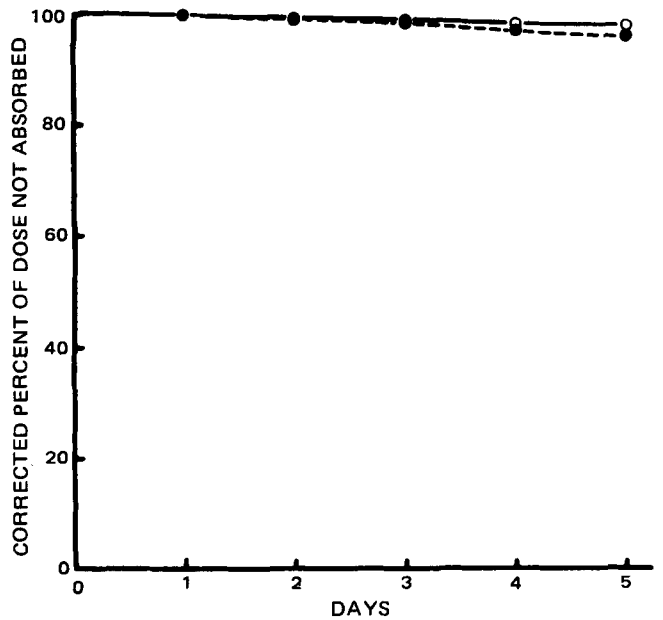

Figure 1—*Percutaneous absorption of hydrocortisone in humans and guinea pig ear. Key: (○) human (2); (●) guinea pig. All data are corrected for recovery following systemic administration. Guinea pig ear data are also corrected for thickness using Eq. 2.*

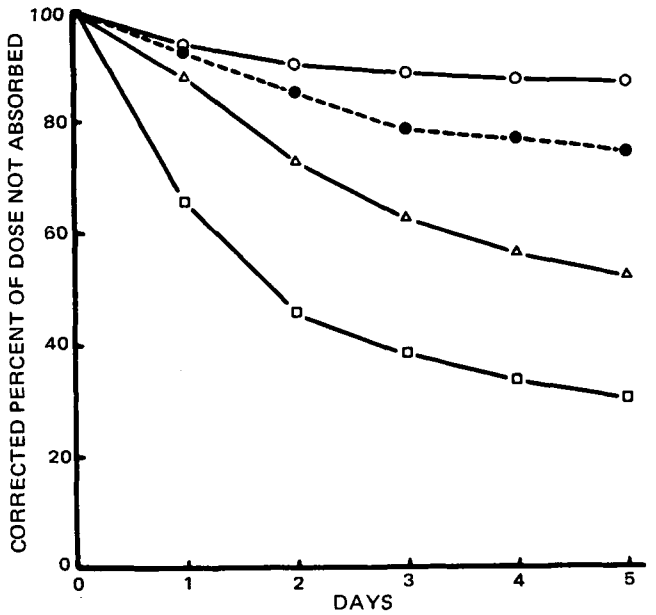

Figure 2—*Percutaneous absorption of testosterone in humans, rats, rabbits, and guinea pig ear. Key: (○) human (2); (●) guinea pig; (△) rat (1); (□) rabbit (1). All data are corrected for recovery following systemic administration. Guinea pig ear data are also corrected for thickness using Eq. 2.*

at the end of its respective 24-hr collection period. Figure 5 shows the relative humidity at the time of urine collection for the hydrocortisone and testosterone experiments. Note in Fig. 4 the drop in the testosterone curve on day 4 and the rise in the hydrocortisone curve on day 5. Corresponding changes in relative humidity can be seen in Fig. 5. It appears that these countertrend changes in percutaneous absorption reflect changes in the ambient relative humidity. Moreover, these changes in relative humidity are also reflected in the volume of urine excreted (Fig.

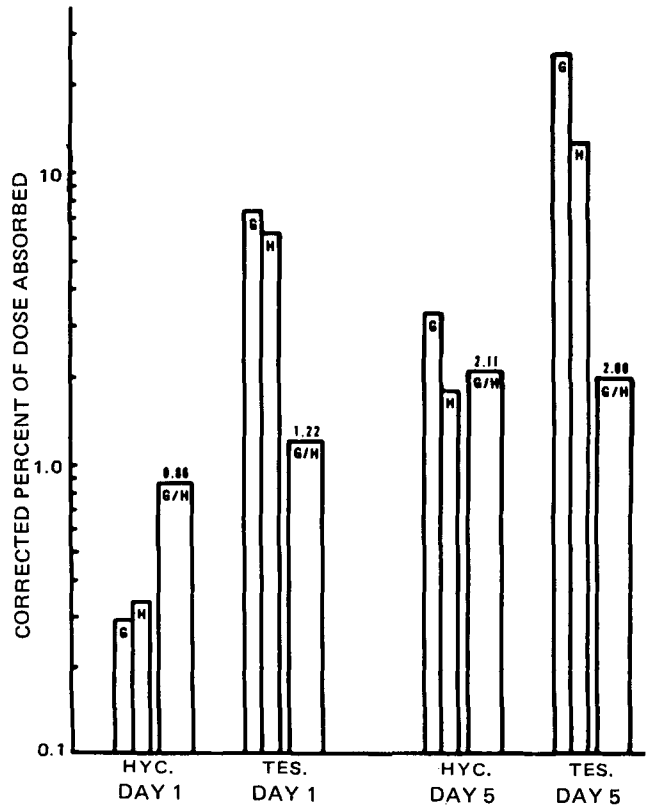

Figure 3—*Comparison of percutaneous absorption in guinea pig ear and humans (2) 1 day and 5 days following topical application. Key: (G) guinea pig; (H) humans.*

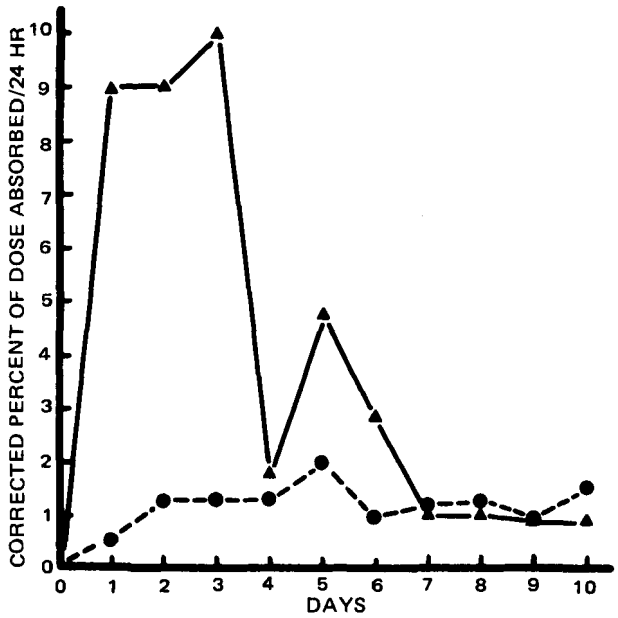

Figure 4—*Percutaneous absorption of hydrocortisone and testosterone over a 10-day period. Key: (▲) testosterone; (●) hydrocortisone. Each point is the average of two animals.*

6) and in the urine color quench (Fig. 7). This can be seen by comparing Fig. 5 with Figs. 6 and 7.

DISCUSSION

To be useful, an animal model should correlate with the desired parameter, and it should be accessible to a large number of researchers. Use of the guinea pig ear as a model for human percutaneous absorption may meet these criteria.

The animal is relatively inexpensive to acquire and house, its ears each have an area of ~3 cm^2, and are essentially hairless. Hairless guinea pig ear skin, as in humans, would be expected to have a thicker stratum corneum than back skin with hair because it must adapt to greater mechanical and temperature stress (1).

Human stratum corneum is on the average of 15 cells thick with a viable layer of 6–10 cells (9, 10). The guinea pig ear skin compares favorably with human skin, having a viable thickness of 7–8 cell layers and a stratum corneum thickness of 10–12 cells (12, 13). In addition, the correlation between epidermal thickness and turnover time (maturation) for human and guinea pig ear epidermises are nearly identical (14). This is important, since the structural organization of normal stratum corneum is dependent upon a slow rate of cell maturation (14).

Percutaneous absorption studies presented here using the guinea pig ear show that for hydrocortisone and testosterone the initial 24-hr period of absorption is comparable to human absorption if a correction is made for differences in stratum corneum thickness. Five-day cumulative absorption in this model is twice as great as in humans. This might be attributed to three possible factors. First, in the human studies (2, 3), the subjects were allowed to wash the application site after the first 24 hr. Thus, some material could have been removed. Second, in our studies no attempt was made to prevent exfoliation of the stratum corneum. It is possible that radioactive squama contamination could have elevated the urine counts. We did not attempt to prevent this because almost any attempt to prevent exfoliation would most likely increase the local moisture content of the stratum corneum. As the relative humidity studies have shown, this can increase absorption. Finally, there may be physical differences between human stratum corneum and guinea pig squama as there may be human differences (15).

In a recent study (16), the guinea pig back skin was used as a model for percutaneous absorption; the much thicker and hairless skin of the guinea pig ear was used as a model in this study. A comparison of the 1-day guinea pig back skin to human forearm absorption ratio (G/H) for hydrocortisone and testosterone (0.30 and 2.23, respectively) showed a much larger variation than the ratios of 0.86 and 1.22, respectively, obtained with the ear model in this report. Similarly, the G/H for the 5-day absorption studies for hydrocortisone and testosterone (1.33 and 3.83, respectively) also showed a much greater variation than the ratios of 2.11 and 2.00, respectively, obtained in this study with the guinea pig ear. The ear skin model would be expected to be a superior model compared to the back skin because the stratum corneum is thicker in accordance with the inverse relationship between stratum corneum thickness and hairiness (9) and the ear is hairless so that no depilation is necessary as it is for the furred back skin.

Hydrocortisone and testosterone were chosen for this study because these compounds are among the slowest and the fastest steroids absorbed by humans. If further studies show that the ratio between the guinea pig ear model and human percutaneous absorption remain constant as they do for these steroids, the guinea pig ear model may prove to be an inexpensive, accessible model for human percutaneous absorption.

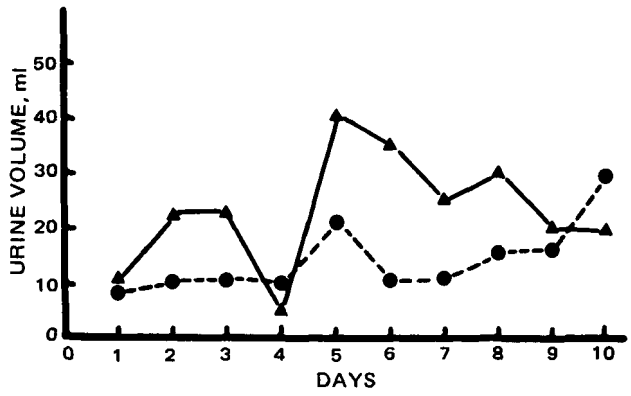

Figure 6—*Volume of urine excreted during 10-day hydrocortisone and testosterone experiments. Key: (▲) testosterone; (●) hydrocortisone.*

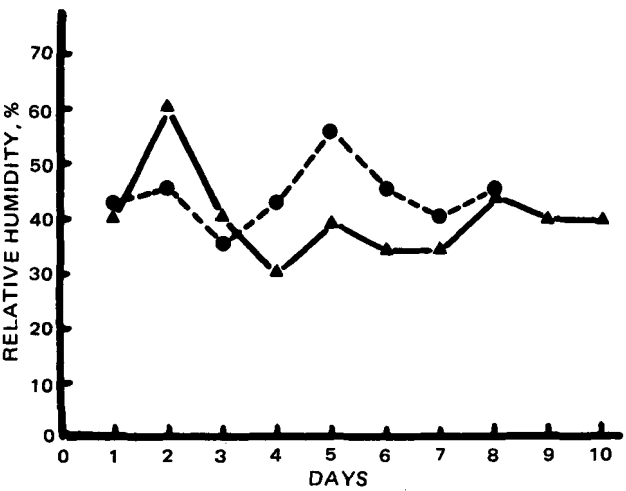

Figure 5—*Relative humidity during 10-day hydrocortisone and testosterone experiments. Key: (▲) testosterone; (●) hydrocortisone.*

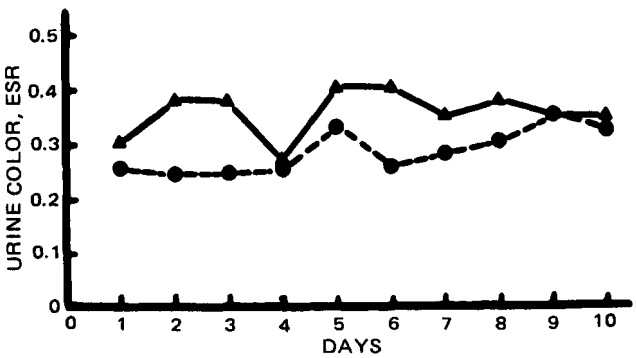

Figure 7—*Urine color during 10-day hydrocortisone and testosterone experiments. Key: (▲) testosterone; (●) hydrocortisone. Urine color was quantified as quench changes in the external standard ratio (ESR) standardization during liquid scintillation counting.*

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Improved High-Pressure Liquid Chromatographic Method for the Analysis of Erythromycin in Solid Dosage Forms

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Abstract □ A stability-indicating high-pressure liquid chromatographic (HPLC) method for the assay of erythromycin in enteric film-coated tablets was developed. The method used a reversed-phase column at 70° with a mobile phase of acetonitrile–methanol–0.2 *M* ammonium acetate–water (45:10:10:35) at pH 7.0. The column effluent was monitored at 215 nm. Several reversed-phase columns were evaluated for the analysis of erythromycin. The HPLC method was also applicable for the analysis of salts and esters of erythromycin. The linearity and precision of the HPLC assay method for erythromycin in the solid dosage form were examined by spiking erythromycin into a tablet placebo at 60–120% of the label. The recovery of erythromycin was 99.9% with a relative standard deviation of <1%. The correction factors to express the results of HPLC in terms of antimicrobial bioequivalency against *Staphylococcus aureus* ATCC 6538P for erythromycins A, B, and C were determined to be 1.0, 0.92, and 0.48, respectively. Eight lots of tablets were assayed by the HPLC method, and the results, expressed in terms of erythromycin bioequivalency, showed no statistically significant difference from those of the microbiological assay method.

Keyphrases □ High-pressure liquid chromatography—improved method for the analysis of erythromycin in solid dosage forms □ Dosage forms—improved high-pressure liquid chromatographic method for the analysis of erythromycin □ Erythromycin—solid dosage forms, improved high-pressure liquid chromatographic method for analysis

Erythromycin, a macrolide antibiotic, is normally administered orally and is marketed in several forms. These include the free base, salts such as the stearate, and esters such as ethyl succinate and 2′-*O*-propionyl. Erythromycin free base is formulated as an enteric coated tablet to protect erythromycin from acid degradation and to allow absorption in the intestinal tract.

High-pressure liquid chromatographic (HPLC) methods for the determination of erythromycin free base and an ester of erythromycin, erythromycin ethyl succinate, have been reported (1–4). The HPLC assay method for erythromycin ethyl succinate uses an elevated column temperature at 70° to minimize peak tailing and to improve peak resolution (4). This HPLC method was adopted for monitoring the clinical blood level of erythromycin and erythromycin ethyl succinate by use of a postcolumn extraction and derivatization technique for fluorimetric detection (5).

Since the HPLC method reported for the assay of erythromycin uses ambient column temperature (1), considerable peak tailing and lack of peak resolution have been experienced. The method reported in this paper uses elevated column temperature for optimum peak resolution and minimum peak tailing for the assay of erythromycin in a solid dosage form.

EXPERIMENTAL

Apparatus—A modular liquid chromatograph equipped with a variable wavelength detector at 215 nm[1], a high-pressure pump[2], and a 100-μl fixed loop injector[3] were used. A reversed-phase HPLC column[4] was water jacketed and maintained at 70° with a circulating water bath[5]. The peak area was electronically determined by use of an electronic integrator[6].

Reagent—All the solvents used were distilled in glass and were UV grade[7]. Ammonium acetate used was analytical reagent grade. The mobile phase composed of acetonitrile–methanol–0.2 *M* ammonium acetate–water (45:10:10:35) was filtered through a membrane filter[8]. The flow rate of the mobile phase was ~1.0 ml/min.

The 0.2 *M* ammonium acetate was prepared by weighing 15.5 g of ammonium acetate[9] into a 1-liter graduated cylinder and adding water to volume.

[1] Model 1201 Spectromonitor I, Laboratory Data Control, Riviera Beach, Fla.

[2] Model 196-0066-02 High-Pressure Mini Pump, Laboratory Data Control.

[3] Model 70-10 Loop Injector, Rheodyne, Berkeley, Calif.

[4] 18-5A, LiChrosorb RP-18, Brownlee Labs, Santa Clara, Calif.

[5] Lauda K-2/R controlled-temperature circulating water bath, Brinkmann, Lauda, GFR West Germany.

[6] Chromatopac-E1A, Shimadzu Seisakusho, Ltd., Kyoto, Japan.

[7] Burdick and Jackson Labs, Muskegon, Mich.

[8] Catalog No. FHLP04700 Fluoropore Filter, Millipore Corp., Bedford, Mass.

[9] Mallinckrodt, Inc., Paris, Ky.

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