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Adsorption of Tuberculin PPD to Glass and Plastic Surfaces

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For some time it has been known that the adsorption of tuberculin to glass is a source of practical difficulties in tuberculin testing; for example, it leads to a loss of potency in diluted tuberculin PPD preparations used in the intracutaneous method of skin testing. The authors have correlated decreasing biological potency with decreasing radioactivity in solutions of tuberculin PPD labelled with ^{14}C.

The decrease in radioactivity is due to adsorption of PPD-^{14}C to the glass or plastic surface of containers; it can be prevented by the addition of 0.0005% Tween 80. The extent of the decrease is affected by the type and size of the containers, the volume of solution used and the storage temperature. It is the same in the presence of 0.3% phenol or 0.01% Chinosol used as preservatives. The concentration of Tween 80 does not affect the size of the tuberculin skin reactions in BCG-sensitized guinea-pigs.

It is recommended that an anti-adsorption agent be added to all dilute solutions of tuberculin PPD; in solutions for intracutaneous use containing 50 TU per ml, Tween 80 at a concentration of 0.0005% is satisfactory.

As early as 1935 it was demonstrated that tuberculin is adsorbed on glass (Parish & O'Brien, 1935). Several investigators have pointed to adsorption as a source of practical difficulties in tuberculin testing. Nelson, Seibert & Long (1937) concluded from their observations that "a certain proportion of tuberculin-positive subjects react to the injection of a physiologic solution of sodium chloride from syringes previously used for tuberculin and then simply washed and sterilized."

More recently, Waaler et al. (1958) estimated the average loss in potency of tuberculin "purified protein derivative" (PPD) solutions containing 5 tuberculin units (TU) per 0.1-ml dose to be 60%. These findings were supported by Marks (1964), who labelled tuberculin PPD with radioactive iodine (^{131}I) and found a 56% loss of radioactivity in the solution. However, since the iodine is not an integral part of tuberculin, such a loss of radioactivity does not necessarily imply the adsorption of PPD to glass. Magnusson et al. (1958) carried out an extensive study to determine whether diluted solutions of tuberculin PPD could be stabilized by using a substance that would prevent the adsorption of PPD to the wall of the container. They concluded that full stability could be obtained by using 0.005% Tween 80.

For some time we have been greatly concerned about the loss of potency in diluted tuberculin PPD preparations employed for the intracutaneous method of skin testing. We decided, therefore, to correlate the decreasing biological potency of PPD solutions, as reported by Magnusson et al. (1958), with decreasing radioactivity in such solutions. Tuberculin was labelled with ^{14}C rather than ^{131}I so that any loss of radioactivity from solution could be unequivocally related to loss of tuberculin.

MATERIALS AND METHODS

Reagents

Phenol (Analytical Reagent grade) was supplied by The British Drug Houses Ltd, Poole, England; Chinosol (8-quinolinol sulfate) by Eastman Organic Chemicals, Rochester, N.Y., USA and Tween 80 (polyoxyethylene (20) sorbitan mono-oleate) by Atlas Chemical Industries, Wilmington, Del., USA.

Buffer solution

Isotonic phosphate-buffered solution (Magnusson et al., 1958; Landi, 1963) pH 7.38, containing 1.45 g

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of KH_2PO_4, 7.60 g of $Na_2HPO_4,2H_2O$ and 4.80 g of NaCl per litre, was used.

Preparation and purification of tuberculin PPD-^{14}C

Tuberculin PPD-^{14}C was produced by adding 200 microcuries of an acid hydrolysate of Chlorella protein-^{14}C (New England Nuclear Corp., Boston, Mass., USA) to 450 ml of Long's synthetic medium (Long & Seibert, 1926). This medium was distributed equally in three 1-litre Roux bottles and seeded with a pellicle of *Mycobacterium tuberculosis* var. *hominis* Johnston strain and incubated at 37°C±0.5°C for six weeks. After their contents had been tested for purity, the bottles were steamed for three hours in a flowing-steam cabinet (100°C) and cooled to room temperature. The bacterial growth was separated from the medium by filtration.

Tuberculin PPD was prepared from the filtrate designated "crude tuberculin solution" by precipitation with trichloroacetic acid (TCA), employing essentially the method described by Landi & Held (1965a). Tuberculin PPD prepared from crude tuberculin solution by TCA-precipitation contains, in addition to tuberculoprotein, a variable amount of nucleic acid. This particular preparation of PPD-^{14}C contained 37.0% by weight of nucleic acid-^{14}C, which accounted for 16% of the radioactivity of the preparation. Since we have established that nucleic acid is not adsorbed to the wall of the containers (unpublished results), the radioactivity due to nucleic acid-^{14}C was deducted from the total radioactivity in solution, in order to obtain the true amount of radioactive tuberculoprotein. More details about the preparation of radioactive tuberculoprotein-^{14}C will be published elsewhere.

Preparation and dispensing of tuberculin PPD-^{14}C solutions

Solutions of tuberculin PPD-^{14}C, with and without Tween 80, were prepared in a buffer solution containing 0.3% phenol and 1 or 0.5 μg of tuberculoprotein per ml as PPD-^{14}C (50 or 25 TU/ml). These solutions were dispensed into vials or ampoules (Table 1) in aliquots of 2.5 or 5 ml. The containers were stored at 10°C-12°C and withdrawn at various time intervals. In a few experiments some containers were also stored at 20°C and 37°C, and 0.3% phenol was replaced by 0.01% Chinosol.

Preparation and dispensing of non-radioactive tuberculin PPD

Tuberculin PPD was prepared as described previously by Landi (1963).

TABLE 1

DIMENSIONS, CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF GLASS VIALS AND AMPOULES

Property	Vial [a]	Ampoule [b]
Dimensions		
Inside diameter (cm)	1.8	1.6
Height: total (cm)	4.7	6.7 [c]
inside [d] (cm)	3.4	4.5
Inner glass surface (cm²)	19.2	24.2
Total volume (cm³)	8.6	10.1
Ratio of inner glass surface to volume [e]		
per total volume	2.23	2.40
per volume filled: 5 ml	3.84	4.84
2.5 ml	7.68	9.68
Chemical composition [a, b]		
(% by weight)		
SiO_2	68.1	73.3
Al_2O_3	5.8	6.7
B_2O_3	10.8	9.5
BaO	3.7	2.2
CaO	0.6	0.8
ZnO	0.9	—
Na_2O	8.7	6.5
K_2O	1.3	0.6
Cl	—	0.2
Physical properties [a, b]		
Density (20°C)	2.463	2.36
Linear coefficient of expansion 0°C-300°C (per deg C)	61×10^{-7}	50×10^{-7}
Chemical durability [f] (ml 0.02-N H_2SO_4)	0.50	0.36

[a] Vial designation (No-Sol-Vit type 1 Glass S-792M) and chemical and physical data were supplied by T. C. Wheaton Co., Milville, N.J., USA.

[b] Ampoule designation (N 51 A break 12012 fl. 2) and chemical and physical data were supplied by Owens-Illinois Inter-America Corp., Toledo, Ohio, USA.

[c] After flame-sealing.

[d] From bottom to lower end of neck.

[e] Since in a closed system, e.g., an ampoule or stoppered vial, the surface above the liquid level is completely covered by a liquid film in which the surface-active substances can spread out, the whole inner glass surface was used for the calculation of these ratios.

[f] According to *United States Pharmacopeia XVI* (1960)—type I test procedure.

Solutions of tuberculin PPD (50 TU/ml), with and without Tween 80, were prepared and dispensed in the same manner as described for PPD-^{14}C solutions.

Containers

Ampoules. Particulars of the ampoules used are listed in Table 1.

Vials. Particulars of the vials are listed in Table 1. All vials were closed with white rubber stoppers (V-32-86, The West Company, Phoenixville, Pa., USA) and sealed with a three-piece lacquered aluminium cap (No. 13-31, The West Company). When Chinosol was used as preservative, the stoppers were pre-equilibrated against a buffered solution containing 0.01% Chinosol (Landi & Held, 1965b). No pretreatment of stoppers was necessary when phenol was used as preservative since the partition coefficient of phenol between rubber and an aqueous solution is small (Wing, 1956).

Syringes. 1 ml B-D Yale tuberculin glass syringes and 1 ml B-D tuberculin plastic disposable syringes (Becton, Dickinson & Co., Rutherford, N.J., USA) were used.

Assays for radioactivity

Aliquots of 1 ml were plated in quadruplicate on stainless-steel planchets and counted with a Geiger-Müller gas-flow counter fitted with a Micromil (Nuclear-Chicago Corp.) window.

Some ampoules were used to determine directly the PPD-^{14}C adsorbed on the glass. This was done by placing the emptied ampoule, which fitted perfectly, in the centre position of the screw-cap bottle (so that the geometry was not altered) of a liquid scintillation counter (Nuclear Chicago, Model 6725), filling the bottle and ampoule with scintillation fluid (3 g *p*-terphenyl + 30 mg POPOP per litre of toluene) and counting the adsorbed PPD-^{14}C.

Biological testing

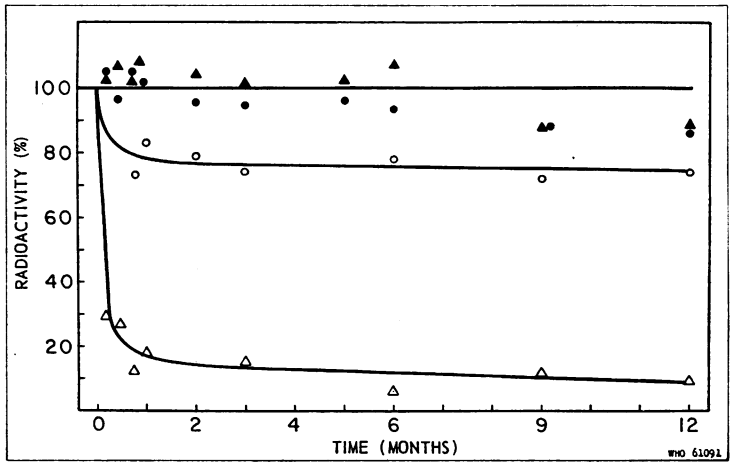
Biological testing was carried out with non-radioactive tuberculin PPD in BCG-sensitized guinea-pigs. Both flanks of each guinea-pig were divided into four squares. Doses were randomized to provide a final design in which each dose appeared in all positions. One-tenth millilitre of the solution under test and containing 50 TU/ml of PPD, with and without Tween 80, and standard solutions containing 25 TU/ml and 100 TU/ml (both with 0.0005% Tween 80) were injected intracutaneously by means of 1-ml tuberculin glass syringes. The control solutions were always freshly prepared. The results were analysed statistically.

RESULTS

Adsorption of tuberculin PPD-^{14}C by determining the loss of radioactivity in solution

Adsorption of tuberculin PPD-^{14}C to glass. Vials and ampoules each containing 2.5 ml or 5 ml of a 50 TU/ml solution of PPD-^{14}C, with and without Tween 80, were stored at 10°C-12°C for 12 months. Fig. 1 shows that no appreciable loss of radioactivity occurred in the presence of Tween 80 (0.0005%), whereas in its absence the maximum loss of radioactivity was approximately 25% for the vials

FIG. 1
EFFECT OF TWEEN 80 ON ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS VIALS AND AMPOULES DURING STORAGE AT 10°C-12°C [a]

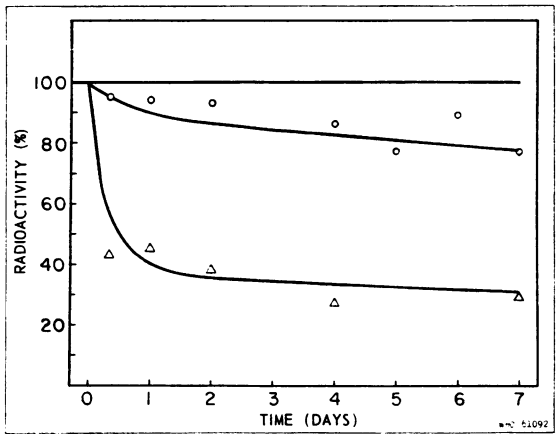


- ○ Vials; no Tween 80
- ● Vials; 0.0005% Tween 80
- △ Ampoules; no Tween 80
- ▲ Ampoules; 0.0005% Tween 80

[a] Vials and ampoules contained 2.5 ml of solution (50 TU/ml; 0.3% phenol).

FIG. 2

ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS VIALS AND AMPOULES DURING STORAGE AT 10°C-12°C [a]



o Vials Δ Ampoules

[a] Vials and ampoules contained 2.5 ml of solution (50 TU/ml; 0.3% phenol) without Tween 80.

and 90% for the ampoules. It was also found that in the ampoules a loss of about 50% in radioactivity took place within eight hours (Fig. 2), whereas little loss took place in the vials during the same period. A comparison of Fig. 1 and Fig. 3 shows that in vials and ampoules containing 5 ml of PPD-^{14}C solution (50 TU/ml) instead of 2.5 ml, the average loss of radioactivity was reduced.

From these results it is evident that the type and size of the container, as well as the volume of PPD solution, are important factors influencing the loss of radioactivity from the tuberculin PPD solution The loss of radioactivity from the solution indicates that PPD-^{14}C was adsorbed on the glass surface. This was demonstrated by shaking emptied vials with a fresh buffer containing 0.005% Tween 80 and making subsequent ^{14}C assays of the solution, and by direct measurement of the ^{14}C on the inner glass surface of emptied ampoules, using a liquid scintillation counter.

Determination of minimum amount of Tween 80 required to prevent adsorption of tuberculin PPD to the wall of glass containers

Ampoules. Ampoules were chosen for this experiment because the rate of adsorption of tuberculin PPD-^{14}C to the walls was found to be much greater for this type of container than for vials. Different concentrations of Tween 80 were used —0.0005%, 0.00005%, 0.000005%—and a solution containing no Tween 80 was employed as control. Fig. 4 shows that there was no appreciable loss in radioactivity from the solution containing 0.0005% Tween 80, whereas a 50% loss occurred in the solution containing 0.00005%. The lowest concentration of Tween 80 was without effect. Therefore a concentration of 0.0005 % Tween 80 is sufficient to prevent the adsorption of tuberculin PPD onto the glass surface of the ampoules over a period of 12 months at 10°C-12°C. This concentration is one-tenth of that recommended by Magnusson et al. (1958).

Vials. Although the use of ampoules has proved an excellent tool for investigating the adsorption of

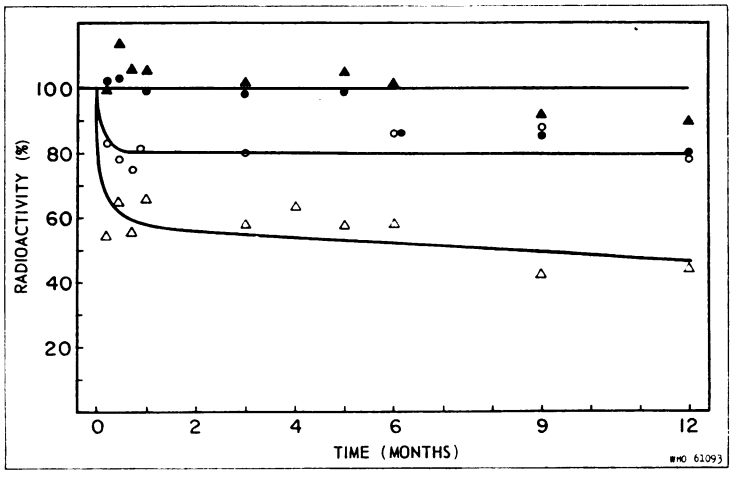


FIG. 3

EFFECT OF TWEEN 80 ON ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS VIALS AND AMPOULES DURING STORAGE AT 10°C-12°C [a]

o Vials; no Tween 80

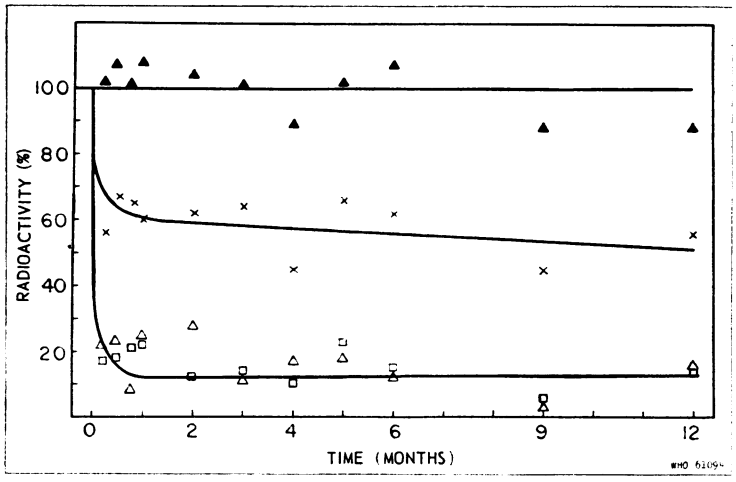
• Vials ; 0.0005 % Tween 80

Δ Ampoules; no Tween 80

▲ Ampoules ; 0.0005 % Tween 80

[a] Vials and ampou cseiontained 5 ml of solution (50 TU/ml; 0.3 % phenol).

FIG. 4
EFFECT OF TWEEN 80 ON ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS AMPOULES DURING STORAGE AT 10°C-12°C [a]



Tween 80 concn
▲ 0
□ 0.000005 %
× 0.00005 %
▲ 0.0005 %

[a] Ampoules contained 2.5 ml of solution (50 TU/ml; 0.3 % phenol).

PPD-^{14}C to glass, one should remember that our tuberculin PPD is dispensed in a multidose vial and, as shown above, the loss of radioactivity from solutions dispensed in this type of glass container is much less than from those dispensed in ampoules. In fact, Fig. 5 shows that adsorption of tuberculin PPD to glass vials could be almost entirely prevented over a period of 12 months with even as little as 0.00005 % Tween 80.

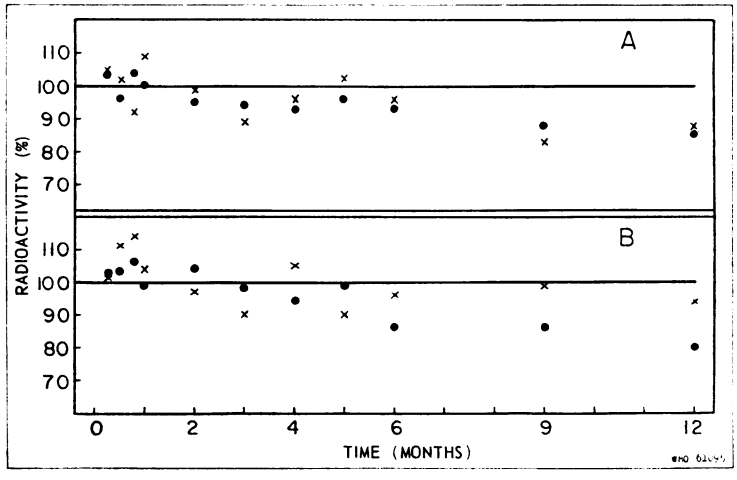
Adsorption of tuberculin PPD drawn into 1-ml syringes. Table 2 shows that when 1 ml B-D Yale tuberculin glass syringes and 1 ml B-D tuberculin plastic disposable syringes are filled with 1 ml of a freshly prepared tuberculin solution (25 TU/ml), about 20 % of the PPD-^{14}C is adsorbed to the glass

TABLE 2. ADSORPTION OF TUBERCULIN PPD-^{14}C (25 TU/ml; 0.3 % PHENOL; NO TWEEN 80) DRAWN INTO 1 ml B-D TUBERCULIN SYRINGES

Time in syringe (min)	Loss of radioactivity in solution (%) [a]	
	Glass syringe	Plastic syringe
4	0	6
8	8	7
12	5	12
16	23	19
20	23	22
24 hours	48	84

[a] 1 ml solution was withdrawn into syringes from one-litre Pyrex-Erlenmeyer flask containing 250 ml of tuberculin PPD-^{14}C solution.

FIG. 5
ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS VIALS DURING STORAGE AT 10°C-12°C [a]



• 0.0005 % Tween 80
× 0.00005 % Tween 80

[a] Vials contained 2.5 ml (A) or 5 ml (B) of solution (50 TU/ml; 0.3 % phenol).

TABLE 3
EFFECT OF TWEEN 80 ON ADSORPTION OF TUBERCULIN PPD-^{14}C IN 1-ml B-D TUBERCULIN SYRINGES

Tween 80 concn	Loss of radioactivity in solution (%) [a] in			
	Glass syringes		Plastic syringes	
	after 20 min	after 24 h	after 20 min	after 24 h
Nil	10	72	20	80
0.00005%	10	64	24	61
0.0005%	5	14	1	5

[a] 1 ml solution was withdrawn into syringes from vials containing 5 ml tuberculin PPD-^{14}C solution (50 TU/ml) that had been stored for 9 months at 10°C-12°C.

or plastic walls of the syringes after 20 minutes of contact, while after 24 hours 48% and 84% of the PPD is adsorbed to the glass and plastic surfaces, respectively.

Since Tween 80 at a concentration as low as 0.00005% can prevent the adsorption of tuberculin PPD to glass vials (Fig. 5), an experiment was designed to determine if this low concentration of Tween 80 will also prevent the adsorption of tuberculin to glass and plastic syringes when the tuberculin solution is withdrawn from vials. Tuberculin PPD-^{14}C solutions (5 ml per vial and 50 TU/ml) without Tween 80 or containing 0.00005% or 0.0005% Tween 80, and stored for nine months at 10°C-12°C, were used. One-ml aliquots of each of these solutions were withdrawn into 1-ml glass or plastic syringes. Table 3 shows that, after 20 minutes of contact with the glass syringes, the solution of tuberculin containing no Tween 80 lost 10% by adsorption, whereas 20% adsorption took place in the plastic syringes. After 24 hours the adsorption was 72% and 80%, respectively. When the solution of tuberculin containing 0.00005% Tween 80 was used the results were similar, although the loss by adsorption was slightly reduced. However, in presence of 0.0005% Tween 80 practically no adsorption occurred.

Effect of temperature on loss of tuberculin PPD-^{14}C by adsorption to glass vials during storage

Vials filled with 2.5 ml or 5 ml of a 50-TU/ml solution of PPD-^{14}C, without and with Tween 80 (0.001%), and 0.3% phenol were stored at 10°C-12°C, 20°C and 37°C. Samples were taken for ^{14}C assays after three and 12 months' storage.

Table 4 shows that after three months' storage the loss of radioactivity for preparations with and without Tween 80 was not significantly different at the three temperatures. After 12 months' storage, however, the loss of radioactivity was higher at 20°C and 37°C than at 10°C-12°C.

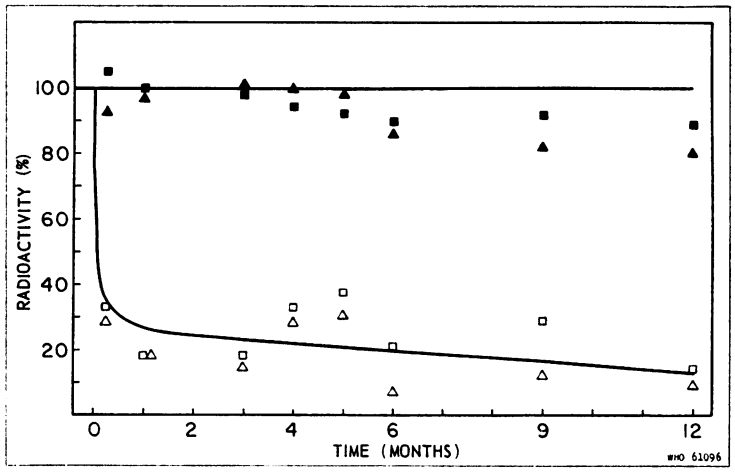
Adsorption of tuberculin PPD-^{14}C to glass in presence of Chinosol

Since Chinosol has been extensively used in tuberculin solutions as an antimicrobial agent (Magnusson et al., 1958), it was of interest to determine if the replacement of phenol by Chinosol in

TABLE 4
EFFECT OF TEMPERATURE ON LOSS OF TUBERCULIN PPD-^{14}C BY ADSORPTION TO GLASS VIALS DURING STORAGE

Volume of solution in vial (ml)	Loss of radioactivity in solution (%) at					
	10°C-12°C		20°C		37°C	
	after 3 months	after 12 months	after 3 months	after 12 months	after 3 months	after 12 months
Solutions without Tween 80						
2.5	26	26	34	44	28	46
5	20	22	23	36	12	25
Solutions with 0.001% Tween 80						
2.5	7	7	6	14	4	22
5	10	7	6	11	1	14

FIG. 6
ADSORPTION OF TUBERCULIN PPD-^{14}C TO GLASS AMPOULES DURING STORAGE AT 10°C-12°C IN PRESENCE OF PHENOL OR CHINOSOL [a]



Solutions containing 0.3 % phenol:
△ no Tween 80
▲ 0.001 % Tween 80
Solutions containing 0.01 % Chinosol:
□ no Tween 80
■ 0.001 % Tween 80

[a] Ampoules contained 2.5 ml of solution (50 TU/ml).

these solutions would alter the loss of PPD by adsorption to glass.

Two sets of ampoules filled with 2.5 ml of a 50 TU/ml solution of PPD-^{14}C, without and with Tween 80 (0.001 %), were stored at 10°C-12°C. One set contained 0.01 % Chinosol, the other 0.3 % phenol. Fig. 6 shows that the rate of adsorption of tuberculoprotein to glass is the same in the presence of phenol or Chinosol over a period of 12 months.

BIOLOGICAL TESTING IN GUINEA-PIGS

By using PPD-^{14}C we have confirmed that tuberculin PPD is readily adsorbed to glass and plastic. The following tests were carried out to correlate the loss by adsorption of PPD-^{14}C with the decrease in biological potencies of non-radioactive PPD solutions and to study the effect of Tween 80 on the size of the skin reaction.

Tuberculin PPD (2.5 ml) dispensed in ampoules

Tuberculin skin tests were carried out on 16 BCG-sensitized guinea-pigs, using non-radioactive tuberculin PPD. Table 5 shows that within one day (24 hours) the potency of the PPD solution containing no Tween 80 decreased from 4.77 to 1.30 TU per dose, and after 14 and 84 days' storage the potency was 0.58 and 0.43 TU per dose, respectively. No significant drop in potency occurred in the solution containing Tween 80.

Tuberculin PPD (5 ml) dispensed in vials

Tuberculin skin tests were carried out on 24 BCG-sensitized guinea-pigs, using non-radioactive tuberculin PPD. A scheme similar to that described earlier (p. 595) was followed. No loss of potency was found after eight months, in presence or in absence of Tween 80 (Table 6). The result is in agreement with that of an earlier experiment (Fig. 3), which showed only a small loss of PPD-^{14}C by adsorption to the glass surface of vials containing 5 ml of PPD solution.

TABLE 5
BIOLOGICAL STUDIES ON STABILITY OF TUBERCULIN PPD, WITH AND WITHOUT TWEEN 80, DISPENSED IN GLASS AMPOULES

Storage time (days)	Relative potency in TU [a] of tuberculin PPD solution (50 TU/ml; 0.3 % phenol) containing	
	no Tween 80	Tween 80 (0.0005 %)
0 (approx. 1 h)	4.77 (3.68-6.18) [b]	6.46 (4.97-8.39) [b]
1	1.30 (0.79-2.15)	4.16 (2.62-6.76)
4	1.37 (1.06-1.79)	5.05 (3.96-6.45)
14	0.58 (0.42-0.79)	4.40 (3.36-5.76)
84	0.43 (0.27-0.69)	4.03 (3.29-4.93)

[a] The relative potencies were determined by comparing the solutions being tested with freshly prepared solutions of the same lot of tuberculin PPD containing 0.0005 % Tween 80. Syringes used to inject BCG-sensitized guinea-pigs were precoated with 0.005 % Tween 80 in buffered solution containing 0.3 % phenol.

[b] The values between brackets represent 95 % confidence limits.

TABLE 6. BIOLOGICAL STUDIES ON STABILITY OF TUBERCULIN PPD, WITH AND WITHOUT TWEEN 80, DISPENSED IN GLASS VIALS

Storage time (months)	Relative potency in TU [a] of tuberculin PPD solution (50 TU/ml; 0.3 % phenol) containing	
	no Tween 80	Tween 80 (0.0005 %)
0 (approx. 1 h)	4.95 (3.70-6.60) [b]	6.05 (4.55-8.15) [b]
3	4.90 (3.60-6.55)	4.65 (3.45-6.25)
5	4.15 (3.20-5.40)	4.65 (3.60-6.05)
6	5.10 (3.80-6.85)	6.00 (4.45-8.00)
8	4.05 (2.95-5.52)	6.30 (4.45-8.70)

[a] The relative potencies were determined by comparing the solutions being tested with freshly prepared solutions of the same lot of tuberculin PPD containing 0.0005 % Tween 80. Syringes used to inject BCG-sensitized guinea-pigs were precoated with 0.005 % Tween 80 in buffered solution containing 0.3 % phenol.

[b] The values between brackets represent 95 % confidence limits.

Effect of Tween 80 on size of tuberculin reactions

Many authors (Wijsmuller, 1961; Wijsmuller, Magnusson & Larsen, 1962; Comstock et al., 1964; Guld & Roelsgaard, 1965; Toman et al., 1965) have claimed that the presence of Tween 80 in tuberculin PPD solutions changes the size of tuberculin reactions. To study this point, Tween 80, at concentrations of 0.05 %, 0.005 % and 0.0005 %, was added to tuberculin PPD solutions containing 50 TU/ml, and doses of 0.1 ml were injected at random in eight positions on the back of each of 16 BCG-sensitized guinea-pigs. Table 7 shows that no significant difference in the size of the skin reactions was found between the solutions containing different amounts of Tween 80.

TABLE 7. EFFECT OF TWEEN 80 ON SIZE OF TUBERCULIN REACTIONS IN BCG-SENSITIZED GUINEA-PIGS

Ratios of Tween 80 concentrations in tuberculin solutions (50 TU/ml; 0.3 % phenol)	Ratios of relative potencies [a]
0.05 %/0.0005 %	1.010 (0.798-1.278) [b]
0.005 %/0.0005 %	0.860 (0.680-1.088)
0.05 %/0.005 %	1.176 (0.929-1.488)

[a] Syringes used to inject BCG-sensitized guinea-pigs were not precoated with Tween 80.

[b] The values between brackets represent 95 % confidence limits.

DISCUSSION AND CONCLUSIONS

We have successfully used PPD-^{14}C to establish that the loss of tuberculin PPD from a solution containing no anti-adsorption agent was due to adsorption of tuberculoprotein onto the glass surface of the vials or ampoules into which tuberculin PPD solutions are dispensed. It was also found that the loss by adsorption depends on the time of storage, on the type and size of the container and on the ratio of the volume of PPD solution to the total inner glass surface area of the container. These factors, which contribute to variation in adsorption, must be taken into account in evaluating the potency of a tuberculin preparation, for it is obvious that different pharmaceutical firms will use different types and sizes of glass containers, and unless an anti-adsorption agent is added to the tuberculin PPD solutions no reliable tuberculin PPD can be made available to the users. Furthermore, tuberculin from solutions containing no anti-adsorption agent is readily adsorbed to glass or plastic syringes used to administer the tuberculin intracutaneously.

The loss of PPD is also affected by the storage temperature. Between 10°C and 37°C the loss of tuberculin PPD in a solution without Tween 80 was not affected by temperature over a period of three months. However, after 12 months' storage the loss of PPD was higher at 20°C and 37°C than at 10°C-12°C. The same trend was observed in tuberculin PPD solutions containing Tween 80 (0.001 %).

The loss of tuberculin PPD during 12 months' storage at 10°C-12°C was the same in the presence of either phenol (0.3 %) or Chinosol (0.01 %) as preservative.

Biological tests in guinea-pigs fully confirm that a dilute solution of tuberculin PPD (50 TU/ml) without anti-adsorption agent loses some of its potency when dispensed in small containers. It is, therefore, imperative that an anti-adsorption agent be added to dilute solutions of tuberculin if sources of error in Mantoux tests are to be avoided.

Magnusson et al. (1958) and Marks (1964) have shown that Tween 80 at a concentration of 0.005 % prevents the PPD from being adsorbed to the wall of the glass containers used. By using PPD-^{14}C we were able to confirm these findings and to show that, for the types of ampoule and vial that we used,

0.0005% Tween 80 was sufficient to prevent most of the adsorption of tuberculin PPD (50 TU/ml) over a period of 12 months at 10°C-12°C. When vials were used, even as little as 0.00005% Tween 80 was sufficient.

We also established that a 100-fold difference in the concentration of Tween 80 did not affect the size of the skin reactions on BCG-sensitized guinea-pigs. This is in agreement with the findings of Magnusson et al. (1958).

The striking difference in loss of tuberculin PPD by adsorption between glass ampoules and glass vials cannot be fully explained at present.

As a result of our findings we propose that an anti-adsorption agent be added to all dilute solutions of tuberculin PPD; also, until a more suitable substance than Tween 80 is found, we advocate that Tween 80, at a concentration of 0.0005%, be used in tuberculin PPD solutions (50 TU/ml) for intracutaneous use.

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RÉSUMÉ

On savait déjà que l'adsorption de la tuberculine sur la surface du verre est une source de difficultés dans la pratique des réactions tuberculiniques; elle entraîne une perte d'activité de la tuberculine PPD diluée utilisée pour les réactions intradermiques dont on a montré expérimentalement qu'elle pouvait atteindre 50%-60%. Les auteurs ont cherché à démontrer cette diminution de l'activité biologique en mesurant la chute de la radioactivité dans des solutions de tuberculine PPD marquée au ^{14}C.

La baisse de la radioactivité est due à l'adsorption du PPD-^{14}C sur la surface en verre ou en plastique des récipients: après 8 heures, en l'absence de Tween 80, elle a été d'environ 50% dans les ampoules de tuberculine, mais est restée très faible lorsque la solution était conservée en flacons. Les valeurs maximales de perte de la radioactivité notées dans les solutions conservées en flacons et en ampoules ont été respectivement de 25% et 90%. Le phénomène d'adsorption a pu être démontré directement. Après avoir été vidés de leur contenu marqué au ^{14}C, les flacons ont été remplis d'une solution tampon contenant du Tween 80, dont on a pu mesurer la radioactivité. De même, la présence du ^{14}C a été décelée sur la surface intérieure des ampoules après élimination de leur contenu.

L'adsorption, qui varie selon la taille et le type des récipients, le volume de la solution et la température à laquelle celle-ci est conservée, peut être prévenue par l'addition de Tween 80. Après essais, la concentration optimale du produit semble être de 0,0005% si la tuberculine est conservée en ampoules. En flacons, une teneur en Tween 80 de 0,00005% suffit à empêcher pratiquement toute adsorption de tuberculine sur les surfaces pendant 12 mois. L'addition de Tween 80 à la concentration de 0,0005% aux solutions prévient également le phénomène dans les seringues et notamment les seringues en plastique. L'adsorption ne varie pas en présence de 0,3% de phénol ou de 0,01% de Chinosol utilisés comme antimicrobiens.

La perte d'activité des solutions de tuberculine sans Tween 80 a été confirmée par des réactions biologiques sur cobayes. La concentration du Tween 80 ne modifie pas la taille des réactions cutanées à la tuberculine chez des cobayes sensibilisés par le BCG.

Les auteurs recommandent d'ajouter un agent anti-adsorption à toutes les solutions diluées de tuberculine PPD; le Tween 80 donne de bons résultats s'il est incorporé à la concentration de 0,0005% aux solutions pour usage intradermique contenant 50 UT par ml.

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