TOXICOLOGY CHEMOTHERAPY GBL MICROBIOLOGY

Gibraltar Biological Laboratories, Inc.

23 JUST ROAD, FAIRFIELD, NEW JERSEY
07006
TELEPHONE (201) 227-6882

REPRINTS

Microbiological studies on pressed powders and the sterilizing effect of gamma irradiation

By Herbert N. Prince, Ph.D., and Martin A. Welt, Ph.D.,
Gibraltar Biological Laboratories, Inc., Fairfield, N.J.,
and Radiation Technology, Inc., Rockaway, N.J.

The U.S. Food and Drug Administration has expressed repeated concern over the microbial adulteration of cosmetics and raw materials.[1-3] Findings from the various FDA laboratories, especially the Division of Microbiology in Washington and the New York District laboratories in Brooklyn, have indicated that approximately 20 to 30 percent of cosmetic creams, lotions and skin cleansers were adulterated with high numbers of bacteria, or contained potential pathogens. No extensive data have been reported on the microbiology of nonliquid cosmetics, particularly eye shadows, eye liners, and pressed facial powders, nor on the effects of gamma irradiation to control contamination. The present report contains data bearing on these subjects.

The industrial utilization of radiation sterilization processes has grown markedly during the past decade, especially for use in the field of medical disposable products, and gives evidence of reaching maturity during this decade.[4] There are at least 25 commercial radiosterilization facilities in operation around the world. By far, the principal industrial source of radiation for sterilization appears to be gamma rays from radioisotopic sources such as cobalt-60 or cesium-137. The trend away from electron accelerators and toward "gamma irradiators" for these applications is due in part to the high penetrating power of the gamma ray which permits treating bulky materials within their shipping containers, along with the intrinsic reliability of a mathematically predictable radiation source.

The sterilizing power of gamma radiation is caused by the interaction of the gamma rays with electrons of the target/substrate combination. These interactions, most notably the photoelectric effect and compton scattering, give rise to mobile secondary electrons which travel a relatively short distance but are much more ionizing than the primary gamma ray. In fact, in most cases the gamma ray is responsible for only a small percentage of the total ionization. It is the passage of these ionizing electrons in the vicinity of the microbial species that brings about the inactivation or destruction of the organism. Since only a small temperature rise of the order of a few degrees centigrade accompanies the radiosterilization process, it is referred to as "cold sterilization." It is also important to note that no measurable quantity of radioactivity can be induced in any irradiated product, regardless of quantity, by gamma rays from either a cobalt or cesium source.

Materials and methods

Aerobic plate counts. Pressed powders and raw materials under quality control quarantine were submitted from various manufacturers in sealed plastic bags. Raw material jars were opened aseptically and the upper 10 grams removed and discarded to eliminate surface contaminants. As per the FDA method,[5] 10 grams of material were added to 90 ml of buffered (Tween 20) azolectin diluent (BTA) and appropriate 10-fold serial dilutions made therefrom. Trypticase soy agar pour plates were made in duplicate for each dilution and incubated at 35° C for 48 hours.

Gram negative rod and staphylococcal enumerations. Enrichment cultures were performed on each sample by inoculation of 1.0, 0.1, and 0.01 gram aliquots into Tryptone-Tween-Azolectin (TAT) broth[5] followed by incubation at 35° C for 48 hours with periodic shaking. After primary enrichment, subcultures were made onto the surface of trypticase soy, blood, MacConkey, SS, Pseudosel, and Vogel-Johnson agars for sterility and differentiation of

gram negative rods and pathogenic cocci. Routine biochemical, serological, and microscopic tests were employed for the identification of gram negative rods and gram positive cocci as described elsewhere.[5,6] Hugh's and Leifson's open and sealed O-F medium was used for categorization of all gram negative rods as nonfermenters.

Animal infections. New Zealand white rabbits weighing approximately 2 Kg were employed in experiments designed to simulate in-use ocular infection with eye shadows containing in the order of 10^6 viable pseudomonads per gram. One ml each of overnight broth cultures of *Pseudomonas aeruginosa* B (courtesy of Dr. E. Grunberg, Hoffmann-La Roche, Inc.) and *Pseudomonas stutzeri* (isolated from a batch of FDA certified D & C red azo dye, code name GBL-70-219) were added to 10 grams of sterile (2.5 megarads of gamma irradiation) loose white eye shadow powder having the following composition in % w/w: talcs, 90; zinc stearate, 5; lubricant, 5. A homogeneous paste of culture-eye shadow mixture was made by titration in a sterile mortar; immediately thereafter plate counts were made to determine the number of viable pseudomonads per gram of eye shadow. Nine rabbits were divided into the following groups: a) 3 infected with *P. aeruginosa* eye shadow; b) 3 infected with *P. stutzeri* eye shadow; and c) 3 infected with noncontaminated eye shadow.

Infections were attempted in the following manner: a) rabbits were anesthetized with sodium pentabarbital (50 mg/kg subcutaneously); b) a sterile swab previously rolled in the contaminated or control eye shadow so as to remove 50 to 100 mg, was rubbed onto the corneal surface of both eyes to simulate a mild abrasion under extremely unlikely in-use conditions, as well as onto the upper and lower conjunctival membranes; c) the eyes were held closed for 1 minute after which time the animals were returned to their cages. Animals were observed daily for signs of redness, discharge, iritis, chemosis, corneal opacity, and corneal ulceration. In addition, cultures were taken employing blood, MacConkey, cetrimide, and SS agars.

Irradiation of red D & C azo pigment (GBL-70-219). One hundred gram aliquots of contaminated pigment were placed into polyethylene bags or sealed jars for exploratory irradiation at the following dose levels (in megarads*): 5.0, 2.5, 1.5, 1.0, 0.5, 0.2. The irradiations were carried out on the Radiation Technology, Inc., process irradiator which had been programmed for operation in a batch mode. When in operation the source plaque composed of many high specific activity cobalt-60 pencils is remotely raised from its storage pool to the irradiate position within a concrete labyrinth. The source can then be remotely lowered into the storage pool upon the completion of the prescribed irradiation period.

The samples to be irradiated were placed at points within the cell which had previously been dose mapped, using both Fricke ferrous sulphate and ceric sulphate dosimeters.[7] Both of these chemical systems have been adopted as primary standards for measuring doses in the range of up to 30,000 rads and 2 million rads respectively. Determinations are made spectrophotometrically by determining the ferric ion or cerous ion concentrations. As a secondary check, use was made of a red perspex (dyed plexiglass) system composed of pellets ⅜" diameter by ⅜" long. Under gamma irradiation the pellets will darken and can be interrogated on a digital optical density read-out device. The procedure followed called for the placement of all samples in the cell initially and for their removal periodically until the last batch absorbed the maximum of 5.0 megarads. The dose rate for all irradiations was about 0.45 Mrad/hr.

Visual identification of variances in post-irradiation pigment strength and quality. Top tone and draw-down (rubber spatula) observations were made of the pure color on a white background at all irradiation levels. In addition, pigment was diluted to 10% (w/v) and 50% (w/v) in a suitable organic solvent followed by visual observation in test tubes. Two drops of suspension were then placed on white filter paper, allowed to dry, and observed for color differences. Finally, a 2%, 10%, and 50% dilution (w/v) of each pigment sample in talc was mixed, pulverized, and then observed in the same manner as for the top tone and draw-down tests of pure pigment. The irradiated and control samples thus treated were coded and examined for strength and quality by a panel of 10 cosmeticians known to have normal to excellent color discernment, as judged by standard color evaluation methods (Pseudo Isochromatic Plates—American Optical Company—1965).

Irradiation of FD&C Red No. 3. Approximately 100 milligram samples of FD&C Red No. 3 (2,4,5,7-tetraiodofluorescein) were irradiated in the cobalt-60 facility to doses of 0.2, 0.5, 1.0, and 1.5 megarads. Using a Mettler H10 balance, distilled water solutions were prepared with resultant concentrations ranging from 0.002 to 0.02 mg/ml. Concurrent with this study solutions of FD&C Red No. 3 of the approximate concentrations used above were irradiated at the same dose levels. All the irradiated solutions and the solutions prepared from the irradiated dry powder were compared with controls by measuring the absorbency on a Shimadzu QV-50 spectrophotometer. All readings were made at a slit width of 0.0175 mm and at a wave length of 525 mμ. In order to obtain some indication of the effect of heat on the color fastness of FD&C Red No. 3 in solution, absorbency data were obtained for the same range of concentrations used above, following exposure to a temperature of 120° ± 2°C for 5 minutes.

Although FD&C Red No. 3 was not used as part of this microbiological investigation, its characteristics under irradiation were studied due to some recent interest in this pigment. It had been reported[8] that the FDA Division of Colors and Cosmetics found a 15% destruction of FD&C Red No. 3 following an absorbed cobalt-60 gamma dose of 0.2

* A megarad (1 million rads) equals 10^6 ev absorbed energy/gm of product.

Mrad in a 0.1% solution, while a dose of 1.0 megarad destroyed about 60% of the pigment in solution.

Results and discussion

Pressed powders. A total of 324 samples were tested. From a microbiological point of view these formulations were extremely sound, 233/324 (72%) being negative by both plate count (<300/gram) and the FDA enrichment technique (no gram negative rods or gram positive cocci or yeasts). Within this large microbiologically negative group 20% contained organic pigments and only 5% contained preservatives. All samples were from plants that adhered to good manufacturing practices in respect to quarantine of raw materials, routine cleaning of plant and equipment, and maintenance of hygienic standards among personnel.

A total of 91/324 (28%) of the samples were regarded as contaminated in that they had plate counts of 300 or more bacteria/gram and/or contained mesophilic gram negative rods. A summary of these findings is shown in Table I.

Table I. Percent of pressed powders judged to be microbially contaminated[1] in respect to conventional plate count and FDA analysis.

Plate count[2]	Enrichment culture[3]	No. positive/ No. tested	Tptal positive
+	+	26/324 (8%))	
+	-	4/324 (1%))	91/324(28%)
-	-	233/324 (72%))	
-	+	61/324 (19%))	

[1] = Contaminated not necessarily taken to indicate presence of pathogens
[2] = Positive APC arbitrarily taken at 300/gram or more
[3] = Positive upon isolation after 48 hours of mesophilic gram negative rods, among which were: Ps. putida, Ps. stutzeri, Ps. maltophilia, Herellea vaginicola, Mima polymorpha var. oxidans, (Acinetobacter), Alcaligenes, Achromobacter, Flavobacterium, and Moraxella. No enterobacteriaceae or gram positive cocci were isolated. Ps. aeruginosa was not isolated in this series.

Within this bacteriologically positive group only 8% were positive when the parameters of both high plate count and presence of gram negative rods were employed. This would indicate that GMP and microbiological control, in the absence of preservatives, can materially aid in the formulation of low microbial powders. Of perhaps equal interest is the fact that 19% of the samples contained gram negative rods which could be detected only by extended enrichment at the 1.0 gram as per the FDA method. It should be stressed that any quality control program purporting to have a zero tolerance on gram negative bacilli must routinely employ this type of bacteriological analysis.

With regard to the 27% of samples shown to harbor gram negative rods, only 0.4% were eye liners or eye shadows. Furthermore, with respect to the facial powders, 91% of those containing gram negative rods were formulated with certified organic D & C pigments of various chemical types.

It is not clear why nonfermentative gram negative rods were almost exclusively isolated, especially pseudomonads and members of the tribe *Mimeae.* No enteric bacilli were encountered in this sampling. Equally puzzling is the fact that the predominant mold encountered in this study was *Neurospora crassa.* It is known that these organisms lack the glycolytic enzymes of the Embden-Meyerhoff pathway, and oxidize organic matter by a glyoxylate bypass of the tricarboxylic acid cycle (glyoxylate cycle). Since Zn^{++} stimulates the induction of the enzymes of the glycollate bypass[9] and all of these formations contain substantial amounts of zinc stearate, it is interesting to speculate that the formulations themselves as well as the raw material and manufacturing environment exert some effect upon the recoverable microbial flora.

Raw materials. A summary of the bacteriological results of representative quarantined raw materials is shown in Table II.

Table II. Percent of raw materials judged to be microbially contaminated in respect to conventional plate count and FDA enrichment analysis.

Plate count[1]	Enrichment culture[2]	No. positive/ No. tested	Total positive
+	+	7/55(12%))	
+	-	2/55(4%))	
-	-	36/55(66%))	19/55(35%)
-	+	10/55(18%))	

[1] = Positive taken at 300/gram or more (range = 300 to 100,000 grams)
[2] = Positive for gram negative rods after 48 hour enrichment

Not unexpectedly, contamination profiles of the raw materials closely paralleled those seen for the finished products. We have further analyzed the nature of the microbial flora found in these raw materials and these data are collated in Table III.

Table III. Gram negative rods isolated from various raw material samples.

Raw material	No. Samples Positive/No. S. Tested Mima-Herellea Group	Alcaligenes-Achromobacter-Flavobacterium Group	Pseudomonads
Talcs, U.S.P.	0/10	0/10	1/10
Inorganic pigments[1]	2/35	0/35	3/35
Organic pigments[2]	4/10	3/10	9/10
Zinc stearate, U.S.P.	0/10	0/10	1/10
Mineral oil, light, U.S.P.	0/10	0/10	0/10

[1] = Representative types included: iron oxides (yellow, red, brown), carbon black, titanium dioxide, ultramarine blue, chrome green, manganese violet
[2] = Representative types included: D&C Red No. 7 (calcium lake) and D&C Red No. 9 (barium lake)

As can be seen from data in Table III the organic pigments were the raw materials most frequently associated with the presence of gram negative rods. As with the powders, the organisms isolated were members of the genera *Pseudomonas, Herellea, Mima, Alcaligenes, Flavobacterium,* and *Achromobacter* as well as sporeformers of the genus *Bacillus.* When gram negative rods were detected in the raw materials, *P. stutzeri* appeared 95% of the time.

Organic pigments. Because of the significant influence that certain certified organic D & C pigments seemed to exert upon the microbial quality of these products, a detailed study was undertaken on a sample that was highly contaminated. This sample, designated GBL-70-219, was a red azo dye with an initial aerobic plate count of 10,000/gram. The following studies were undertaken: a) the effect of extended room temperature incubation on viability; b) the ability of gamma irradiation to sterilize this material; and c) the effect of various doses of gamma irradiation upon the visual color properties of the pigment.

Table IV summarizes the qualitative and quantitative changes in flora that resulted from extended room temperature incubation (range of 71° to 78° F).

Table IV. Rise in microbial flora of Pigment GBL 70-219 as a function of room temperature incubation for 6 weeks.

Time (weeks)	Bacteria/gram	Generation time
0	10,000[1]	
1	15,000)	
2	41,200)	84 hours[2]
4	97,000	
6	125,000[3]	

[1] = 60% genus Bacillus, 40% gram negative rods (Ps. stutzeri, Ps. putida, Alcaligenes, Herellea

[2] = This interval taken as period of maximal increase wherein $\log_{10}$ bacteria 2 weeks = $\log_{10}$ at 1 week + n log 2, where n = the number of generations and generation time (g) = t/n, derived from $x = y \times 2^n$

[3] = 70% genus Bacillus plus 30% Ps. stutzeri only

The data in Table IV suggest the presence of an indolent but hardy population of bacteria whose total count had passed the point of exponential multiplication under the conditions studied, but whose arithmetic rate of increase reflected a rate of multiplication in steady state with a slightly lower rate of death. *P. stutzeri* was clearly the most persistent of the organisms encountered, with the exception of the sporeforming bacilli.

At the fourth week a sample of the pigment was exposed to a total dose of 2.5 megarads from a cobalt-60 source. The sample was rendered sterile as judged by sterility tests conforming with USP XVIII and FDA.[5]

A second sample of the same pigment, found to contain 15,000 bacteria/gram, was next exposed to a series of doses of gamma irradiation from a cobalt 60 source. After irradiation a microbiological profile and color characterization as summarized in Table V were obtained.

Table V. Effect of gamma irradiation upon the microbial flora and visual properties of Red D&C Pigment GBL 70-659.

Dose:MR	Bacteria/ gram	Enrichment for gram neg. rods	USP Sterility[1] TSB	Thio	IC	Visual Properties
0	15,000	Ps. stutzeri	+	+	+	A[2]
0.2	ca. 20	Ps. stutzeri	+	+	+	A
0.5	<10	0	0	0	+	A
1.0	<10	0	0	0	0	A
1.5-5.0	<10	0	0	0	0	A

[1] = TSB=trypticase soy broth 10 days at room tempearture; Thio=thioglycollate broth 10 days at 35°C; IC=inoculated carrier (E-Rad-O-Kit[R] Bacillus pumilus spore strip)* 7 days at 35°C

*Available from Gibraltar Biological Laboratories, 23 Just Road, Failfield, N.J. 07006

[2] = A=no statistically significant difference in scoring color variance among panelists, i.e., 5.0 MR indistinguishable from unirradiated control

From the data in Table V it is clear that as low a dose as 0.2 MR produced a 3-log or 99.9% kill, and that 0.5 MR was a sterilizing dose. None of the irradiated samples differed in color from the control to the extent that they could be detected by the human eye, employing dry, moist, and diluted samples.

Of perhaps even greater significance would be information bearing on certain biological properties of irradiated organic pigment. A limited study in these laboratories has shown that an experimental "blush-on" containing irradiated D & C pigment GBL 70-219 (2.5 MR) produced neither skin irritation nor eye irritation in rabbits, nor skin fatigue nor sensitization in guinea pigs, as scored by the conventional Draize test. Additional information bearing on chemical changes and on other biological parameters should be obtained in experimental animals and in volunteers.

Experimental infections. The results of experimental infections under exaggerated in-use conditions are summarized in Table VI.

Table VI. Attempt to induce eye infections in rabbits inoculated with eye shadows intentionally contaminated with Ps. aeruginosa and Ps. stutzeri.

Rabbit	Infection	Eye[1]	Eye rating and culture at day[2] 1	2	3	4	5	6	7	Mean Score[3]	Positive eye Culture
1	E/S+P. aerugin.	L	1*	1*	2*	1*	0	0	0		
		R	1*	1*	1*	1	1	0	0		
2	" "	L	1*	1*	1*	1	0	0	0	1.10	6/6
		R	1*	1*	1*	1*	0	0	0		
3	" "	L	1*	1*	2*	1*	1*	1	0		
		R	1	1	1*	1	0	0	0		
4	E/S+P. stutzeri	L	1	0	0	0	0	0	0		
		R	1	0	0	0	0	0	0		
5	" "	L	1	0	0	0	0	0	0	0.25	0/6
		R	1	0	0	0	0	0	0		
6	" "	L	1	0	0	0	0	0	0		
		R	1	0	0	0	0	0	0		
7	E/S alone	L	1	0	0	0	0	0	0		
		R	0	0	0	0	0	0	0		
8	" "	L	1	0	0	0	0	0	0	0.12	0/6
		R	0	0	0	0	0	0	0		
9	" "	L	0	0	0	0	0	0	0		
		R	1	0	0	0	0	0	0		

[1] = Erythema of eye lid linings and slight chemosis
[2] = Increased redness, chemosis, and discharge
[3] = Sum of numerical values for 1st 4 days divided by total number eye observations for this period per group of 3 rabbits (24)
* = Positive culture

It is clear that the strain of *P. aeruginosa* produced a noninvasive and extremely mild infection characterized by transient conjunctival erythema, slight chemosis, and limited microbial shedding, reaching its peak on the third day post-inoculation. On the other hand, the effects of the *P. stutzeri* and control eye shadows were practically indistinguishable, with no signs of infection apparent by gross observation or by culture.

We hasten to point out that fulminating experimental infections of the rabbit eye with *P. aeruginosa* leading to dendritic keratitis, corneal ulceration, and blindness can only be produced by application of surgical trauma to the cornea followed by massive implanted inoculation.[10] Indeed, infections in man are also usually associated with traumatic corneal abrasion. If these rabbit experiments are taken to simulate in-use contamination of the eye with a pseudomonad-bearing eye shadow, then it would appear that *P. aeruginosa* might in the absence of severe corneal abrasion produce a mild, self-limiting inflammation. We have not detected an experimental model that will indicate the same type of limited pathogenicity for *P. stutzeri.* Further studies with animals and with volunteers would be useful. Until such data are forthcoming it would be prudent to accept zero tolerances for all gram negative rods, especially *P. aeruginosa,* in respect to preparations used in or about the eye.

We are uncertain as to the public health hazard of gram negative rods in general (other than pseudomonads) when they are found at low levels in cosmetic products applied to the unabraded skin of healthy adults. If one avoids the controversial question as to *why* they must be eliminated and addresses oneself to the question as to *whether* they can be eliminated, the problem is soluble. Good manufacturing practices, microbiological testing, and appropriate sterilization procedures all militate

Table VII. Effect of cobalt-60 gamma irradiation on the absorbence characteristics of water solutions of FD&C Red No. 3 pigment.

Form irradiated	Concentration (mg/ml)	Dose (megarads)	Absorbency	Normalized % variation
Solution	0.00200	0	0.184	-
"	0.00400	0	0.340	-
"	0.0100	0	0.890	-
"	0.0200	0	1.88	-
"	0.00200	0.2	0.000	100.0
"	0.00400	0.2	0.019	94.5
"	0.100	0.2	0.150	83.1
"	0.0200	0.2	0.574	69.5
"	0.00200	0.5	0.000	100.0
"	0.00400	0.5	0.000	100.0
"	0.0100	0.5	0.000	100.0
"	0.0200	0.5	0.031	98.2
"	0.00200	1.0	0.000	100.0
"	0.00400	1.0	0.000	100.0
"	0.0100	1.0	0.000	100.0
"	0.0200	1.0	0.005	99.7
"	0.00200	1.5	0.000	100.0
"	0.00400	1.5	0.000	100.0
"	0.0100	1.5	0.000	100.0
"	0.0200	1.5	0.000	100.0
Powder	0.00176	0.2	0.154	nil
"	0.00352	0.2	0.325	nil
"	0.00878	0.2	0.801	nil
"	0.0176	0.2	1.83	nil
"	0.00193	0.5	0.181	nil
"	0.00386	0.5	0.363	nil
"	0.00967	0.5	0.925	nil
"	0.0193	0.5	1.90	nil
"	0.00200	1.0	0.179	nil
"	0.00400	1.0	0.365	nil
"	0.0100	1.0	0.925	nil
"	0.0210	1.0	1.90	nil
"	0.00194	1.5	0.169	nil
"	0.00388	1.5	0.354	nil
"	0.00971	1.5	0.920	nil
"	0.0194	1.5	1.90	nil
Solution	0.00200	0	0.139*	24.5
"	0.00400	0	0.323*	5.0
"	0.0100	0	0.898*	nil
"	0.0200	0	1.90*	nil

* Solutions heated to 120° ± 2° for 5 min.

to make their presence unnecessary.

Table VII summarizes the spectrophotometer data obtained by irradiating FD&C Red No. 3 powder and solutions.

It is clear from the data presented in Table VII that water solutions of FD&C Red No. 3 under cobalt-60 irradiation show a marked loss in color, which corroborates the findings of the FDA. The destruction of pigment is shown to be a function of both the initial concentration and the dose delivered. The situation is quite different for the irradiation of the FD&C Red No. 3 powder prior to incorporation into a water solution. The data shown in Table VII clearly reveal that even under a dosage of 1.5 megarads, which has been found to be quite adequate for the sterilization of most pigments, there is no meaningful deviation in the solution absorbency as compared to the controls.

On the other hand, FD&C Red No. 3 solutions maintained at 120°C for 5 minutes and at the lowest concentrations (0.002 mg/ml) revealed a 10% deviation of the solution absorbency as compared to the controls. Thus, it is observed that heat sterilization can bring about a destruction of the pigment in solution. Similar observations have been made for pigment solutions exposed to UV light or even sunlight. These data help support the claim of the suitability of utilizing gamma ray sterilization on certain organic pigment powders.

Summary

Twenty-seven percent of a series of 324 eye and face powders contained low numbers of gram negative rods. Only 0.4% of these preparations were eye liners or eye shadows. The great majority of these would have been passed as culturally "negative" if plate counts alone were used. Contamination was most frequently traced to D & C organic pigments which contained organisms capable of multiplying within the dry pigment at room temperature. Gamma irradiation at a dose of 0.2 MR produced a 99.9% kill, whereas 0.5 MR rendered a heavily contaminated pigment sterile. Irradiated pigment was not altered in respect to chemical composition, color or certain toxicological parameters. Eye shadows sterilized with cobalt-60 and then intentionally contaminated with pseudomonads induced mild and transient inflammation of the rabbit's eyes in the case of *P. aeruginosa*, but produced no effect in the case of *P. stutzeri*.

Acknowledgements

We wish to thank John Meacham and Henry Sinsabaugh of the FDA District Laboratories, Brooklyn, N.Y., for advice on FDA methods and aid in the identification of certain organisms. Similar thanks are extended to Dr. Carl Bruch, Division of Microbiology, Washington, D.C. Finally, we acknowledge the cooperation of Theodore G. Prince, Laurel Industries Corporation, Elizabeth, N.J., in respect to technical advice in questions relating to cosmetic chemistry.

References

1. Dunnigan, A. P., Microbiological Control of Cosmetic Products. Proc. of Joint Conf. on Cosm. Sci., TGA, 179, 1968.
2. Dunnigan, A. P., and Evans, J., Report on the Special Survey: Microbial Contamination of Topical Drugs and Cosmetics. Am. Perf. & Cosm. **85**, 1, 52, 1970.
3. Clevenger, W., State of the industry: Progress and gaps. Address, N.Y. Chapter, SCC, Rutgers Univ., Oct. 7, 1970.
4. Welt, M. A., Radiosterilizing medical supplies. Disposable soft goods **1**, 4, 10-13, 1970.
5. Microbiological examination of topical drugs and cosmetics. Div. of Microbiology, Bur. Sci., FDA, 1969.
6. Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, 5th ed., Am. Pub. Health Assn., New York, 1970.
7. Hine, G. J., and Brownell, G. L., Radiation Dosimetry. Academic Press, New York, 1956.
8. Clevenger, W., personal communication, June 17, 1971.
9. Wegener, W. S., Reeves, H. C., Rabin, R., and Ajl, S. J., Alternate pathways of metabolism of short-chain fatty acids. Bacterial Reviews **32**, 12-6, 1968.
10. Penn, W. P., Truant, J. P., and Hessburg, P. C., Evaluation of colistin sulfate and the sulfonamides in the in vitro and in vivo treatment of Pseudomonas corneal ulcers. Antimicrobial Agents and Chemotherapy, 411-419, 1963; American Society for Microbiology, 1964.