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A quantitative and qualitative appraisal of microbial pollution of water by swimmers: a preliminary report

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When a person goes swimming, micro-organisms are washed off the skin and out of some body cavities and into the water, polluting it to an unmeasured degree. The normal microbial flora of the human body has been studied by many investigators and has been reviewed comprehensively by Rosebury (1962). The so-called indigenous biota varies from person to person to environment. The organisms, commonly known as pathogens for man, may or may not be present in a healthy person, an individual having a subclinical infection, or a convalescent. All such possibilities have been taken into consideration by public health officials as they have promulgated standards for the bacterial quality of swimming pool waters. Although there has been only limited epidemiologic evidence which has directly related disease with swimming, the possibility is always present.

The lack of these data concerning the disease potential of swimming in polluted water has caused public health and regulatory agencies to use drinking water standards, with some modification, as indices of acceptability. Thus, it has been customary to use chemical disinfectants, usually chlorine or its compounds, in the treatment of swimming pool waters and to apply the same indices of pollution, namely coliform and/or faecal streptococcus groups of bacteria. The use of these indicators has been challenged over the years by many persons, such as Seligmann (1951), Mallman (1928, 1962), and more recently by Favero, Drake & Randall (1964), all of whom believe that cocci represent more correctly the actual bacterial quality of the pool water since large numbers of body cocci are washed into the water. Some of these organisms, such as staphylococci, are moderately resistant to chemical disinfection. McLean (1963) has pointed out that these microbes may be excreted into the water from the skin, and from mouth and nose by sneezing, coughing, and blowing water out of the mouth. Although these practices are discouraged, it is known that they commonly occur. The widespread, almost universal, requirement that a shower be taken before the swimmer dons his suit and enters the pool is intended to reduce the amount of microbial pollution, but the validity of this practice has not been studied thoroughly.

The present study was undertaken to determine, if possible, characteristics of microbiological pollution that a female swimmer might add to filtered water without residual disinfection. This was a preliminary attempt to determine the major types of bacteria being shed under varying conditions of individual personal hygiene, including those during the menstrual period.

MATERIALS AND METHODS

Design of study

A special swimming tank was designed to permit an adult swimmer adequate width and depth to simulate a back stroke, a crawl stroke, and a breast stroke while 'afloat' in 150 gallons of water. The tank was constructed of fibre glass which had been polished to the smoothness of glass and which contained neither seams nor other areas where bacteria might lodge. The design of the tank is shown in Fig. 1.

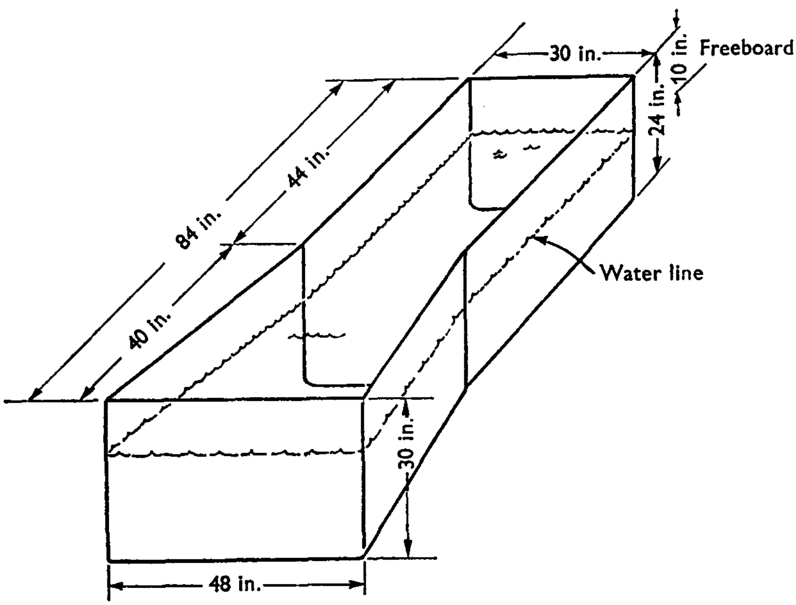

Fig. 1

Water was obtained from the regular city supply. No chlorine residual was ever demonstrated with an amperometric titrator. The water was drawn from a mixing faucet at a temperature of 80° F. through a cartridge-type filter and then directly through nalgene tubing into the experimental tank immediately before each test run.

Five adult females actively engaged in the teaching of physical education, ranging in age from 25 to 45 years, were selected as test subjects. Each individual was asked to maintain her normal daily routine of personal hygiene. Personal data on these subjects are given in Table 1. Four of these individuals took daily tub baths, while one showered daily. Three routinely used brand D soap, one used brand CB, and one used brand I. Three subjects used no skin lotions or special cosmetic preparations; one used brand N, and one used brand P, in daily face washing.

The subjects participated in this experiment during their menstrual period with and without the use of tampons in an attempt to determine whether or not there was any noticeable difference in the bacterial pollution of the water.

When a shower was indicated as part of the experiment, each subject took a hot shower using brand I soap, followed by thorough rinsing. All subjects wore sterile, one-piece cotton bathing-suits and a standard rubber swim-cap; and they wore sterile plastic boots from the locker/shower-room area to the test area.

Table 1. *Personal hygiene data on five female subjects*

Subject	Usual mode of daily bathing	Soap used daily	Skin lotion used	Type of skin	Usual menstrual flow
A	Tub bath	D*	None	Oily	Moderate
B	Tub bath	CB	None	Normal	Moderate
C	Tub bath	D*	N	Normal	Heavy
D	Tub bath	D*	None	Dry	Moderate
E	Shower	I	P*	Normal	Moderate

* Contains hexachlorophene.

The first water sample for bacteriological examination was taken just before the subject entered the tank. The subject, after removing her plastic boots, entered the tank and, supported by a nylon strap, began the series of simulated swimming, 5 min. of the back stroke, 5 min. of the crawl, and 5 min. of the breast stroke.

A sample of water was taken immediately upon conclusion of the swimming period, and a third and final sample was collected 10 min. after the subject had left the tank. All samples were taken at once to the bacteriological laboratory for immediate examination.

Each subject 'swam' a total of ten times, and thus a total of 150 samples were collected and studied.

Bacteriological procedures

All samples were examined for the presence of coliform organisms, faecal streptococci, buccal streptococci, staphylococci, and for total plate counts.

Coliform organisms and faecal streptococci. Both the multiple tube and the membrane filter techniques were used (*Standard Methods for the Examination of Water and Wastewater*, Tenth Edition). The multiple tube test consisted of 3–10 ml., 3–1·0 ml., and 3–0·1 ml. portions of water. All positive presumptive tests were carried through the completed test and the results expressed as Most Probable Numbers (M.P.N.) per 100 ml. of sample. The membrane filter method permitted the filtration of 100 and 10 ml. portions of water, and M-Endo's medium* was used in the detection of coliform organisms and M-enterococcus medium* for isolating the faecal streptococci. All typical colonies were subcultured for complete identification.

Buccal streptococci. Attempts to determine the presence of buccal streptococci were made by passing 100 and 10 ml. portions of water through membrane filters and incubating on 'Mitis-Salivarius' agar.*

* Baltimore Biological Laboratories, Baltimore, Maryland.

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Staphylococci. 10·0 and 1·0 ml. portions of sample were diluted to 100 ml. with sterile phosphate buffer and then passed through membrane filters followed by incubation on Staphylococcus no. 110 agar.*

Standard plate count. Procedures outlined in *Standard Methods for the Examination of Water and Wastewater* (Tenth Edition) were followed, and 1 and 0·1 ml. portions of water were plated, using certified plate count agar.

RESULTS

Control samples

Samples were taken of the filtered water before each subject entered the tank and all bacteriological tests were carried out. No coliform organisms, faecal or buccal streptococci, or staphylococci were ever observed. The standard plate count at no time showed any countable plates. Thus, it has been assumed that although these 50 samples may not have been sterile before each test period, the water did not contain any of the organisms under study in the volumes examined.

Table 2. *Most probable number of coliform bacteria per* 100 *ml. of water sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the M.P.N. 10 min. after the swim.

	Condition of swimmer					
	Not menstruating		Menstruating			
	Shower	No shower	Shower		No shower	
Subject			Tampon	No tampon	Tampon	No tampon
A	9·2 (9·2)	0 (0)	64 (120)	120 (210)	0 (0)	64 (64)
	9·1 (210)	3·6 (9·1)	—	—	0 (0)	—
		120 (9·1)				
B	5·1 (2·2)	> 2400 (2400)	3·6 (9·1)	36 (15)	9·1 (2·2)	64 (64)
	64 (3·6)	240 (120)	—	—	9·1 (5·1)	—
		460 (210)				
C	16 (16)	120 (9·1)	0 (0)	0 (0)	9·1 (3·6)	0 (0)
	0 (0)	0 (3·6)	—	—	0 (0)	0 (0)
D	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		0 (0)	—	—	0 (0)	0 (0)
		0 (0)				
E	9·1 (0)	64 (9·1)	—	—	240 (120)	—
	0 (7·3)	64 (210)	—	—	7·3 (36)	—
	0 (0)	9·1 (120)	—	—	—	—
		43 (93)				
		0 (0)				

Test samples

Coliform determinations

The results obtained by the multiple tube test are shown in Table 2. These results show marked variation in the numbers of coliforms shed by different indivi-

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duals. There is no apparent evidence that any hygienic factor is involved. The results obtained using the membrane filter are given in Table 3 and confirm this observation.

Table 3. *Number of coliform colonies on membrane filter per* 100 *ml. of water sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the counts 10 min. after the swim.

	Condition of swimmer					
	Not menstruating		Menstruating			
	Shower	No shower	Shower		No Shower	
Subject			Tampon	No tampon	Tampon	No tampon
A	0 (0)	0 (0)	37 (31)	55 (60)	0 (0)	4 (50)
	6 (9)	3 (5)	—	—	0 (0)	—
		5 (5)				
B	2 (2)	80 (120)	2 (1)	5 (4)	28 (30)	34 (40)
	9 (7)	19 (17)	—	—	5 (5)	—
		25 (24)				
C	44 (60)	20 (12)	0 (0)	0 (0)	6 (2)	0 (0)
	20 (16)	3 (1)	—	—	0 (1)	20 (10)
D	0 (0)	1 (0)	0 (0)	1 (0)	0 (0)	0 (0)
		2 (0)	—	—	2 (0)	3 (5)
		4 (4)				
E	0 (1)	16 (12)	—	—	2 (0)	—
	3 (2)	90 (170)	—	—	3 (1)	—
	1 (1)	22 (10)	—	—	—	—
		1 (2)				
		140 (70)				

Faecal streptococcus determination

The results of the multiple tube test for demonstrating the presence of faecal streptococci are shown in Table 4 and of the membrane filter method in Table 5. These organisms were not isolated in as great numbers as was the case for coliforms, nor were they always present concurrently. Coliform organisms were present in 28 samples tested by the multiple tube test while faecal streptococci were isolated from only 18 of them. Using the membrane filter method, coliforms were isolated from 39 samples while faecal streptococci were obtained from only 30 of them.

Buccal streptococcus determination

The determination of buccal streptococci was carried out using only 1·0 ml. of the sample and in all probability does not represent an accurate index of the total number. On the medium used (M–S agar) many other micro-organisms grew readily. Strains of *Alcaligenes*, *Bacillus*, *Micrococcus*, *Sarcina*, and yeasts tended to overgrow *Strep. salivarius* when larger quantities of water were filtered. Typical mucoid colonies of *Strep. salivarius* were readily recognized and identified, however, from the 1·0 ml. samples, and it appears to be evident that individuals shed these organisms on many occasions (Table 6).

Table 4. *Most probable number of faecal streptococci per* 100 *ml. of water sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the M.P.N. 10 min. after the swim.

	Condition of swimmer					
			Menstruating			
	Not menstruating		Shower		No shower	
Subject	Shower	No shower	Tampon	No tampon	Tampon	No tampon
A	2·2 (5·1)	0 (9·2)	1100 (1100)	0 (240)	0 (0)	1100 (1100)
	0 (460)	0 (0)	—	—	0 (0)	—
		0 (0)				
B	16 (5·1)	—	64 (64)	64 (9·1)	16 (5·1)	120 (240)
	64 (3·6)	64 (120)	—	—	2·2 (0)	—
		64 (64)				
C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	64 (0)	—	—	0 (0)	0 (0)
D	0 (0)	9·1 (3·6)	0 (0)	0 (0)	0 (0)	0 (0)
		0 (0)	—	—	0 (0)	0 (0)
		0 (0)				
E	1100 (1100)	0 (0)	—	—	0 (0)	—
	0 (0)	64 (64)	—	—	0 (0)	—
	0 (0)	0 (0)				
		0 (0)				
		0 (0)				

Table 5. *Number of faecal streptococcus colonies on membrane filter per* 100 *ml. of water sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the numbers 10 min. after the swim.

	Condition of swimmer					
			Menstruating			
	Not menstruating		Shower		No shower	
Subject	Shower	No shower	Tampon	No tampon	Tampon	No tampon
A	5 (8)	0 (0)	4 (5)	43 (66)	0 (0)	66 (68)
	0 (0)	1 (2)	—	—	0 (0)	—
		0 (0)				
B	34 (40)	38 (33)	6 (3)	6 (8)	15 (22)	32 (30)
	7 (0)	30 (16)	—	—	10 (22)	—
		70 (80)				
C	0 (0)	9 (8)	0 (0)	0 (0)	0 (0)	0 (1)
	1 (3)	36 (27)	—	—	0 (0)	0 (0)
D	4 (1)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		2 (0)	—	—	0 (0)	0 (0)
		3 (2)				
E	0 (9)	0 (0)	—	—	0 (0)	—
	7 (3)	6 (8)	—	—	0 (0)	—
	1 (1)	3 (1)				
		14 (6)				
		66 (70)				

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Staphylococcus detection

Not all the samples collected were studied for the presence of staphylococci. However, all ten samples involving subject E, the last seven involving subject D, and the last six involving subjects A, B, and C were examined for both the presence of staphylococci in general and *Staph. aureus* in particular. The results are given in Table 7, and it is evident that members of this genus were consistently shed in relatively large numbers by all subjects under all conditions. *Staph. aureus* was present in water collected during each experiment in varying numbers, although it was not present in all the samples examined. There did appear to be fewer staphylococci shed by each individual after a shower than when no shower was taken and further study is indicated.

Table 6. *Number of* Streptococcus salivarius *colonies on membrane filter per* 1·0 *ml. of water sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the counts 10 min. after the swim.

	Condition of swimmer					
	Not menstruating		Menstruating			
			Shower		No shower	
Subject	Shower	No shower	Tampon	No tampon	Tampon	No tampon
A	0 (0)	0 (0)	0 (0)	10 (23)	4 (3)	0 (0)
	0 (0)	14 (15)	—	—	0 (0)	—
		0 (1)				
B	8 (6)	0 (9)	0 (0)	8 (15)	0 (0)	8 (9)
	0 (0)	2 (5)	—	—	0 (0)	—
		0 (0)				
C	1 (2)	1 (1)	0 (0)	1 (0)	2 (1)	0 (0)
	0 (0)	1 (2)	—	—	0 (0)	0 (0)
D	3 (1)	36 (7)	0 (0)	0 (0)	0 (0)	1 (0)
		0 (0)	—	—	0 (0)	8 (15)
		0 (0)				
E	15 (2)	20 (10)	—	—	1 (0)	—
	0 (0)	4 (4)	—	—	9 (0)	—
	10 (5)	2 (2)				
		24 (30)				
		10 (8)				

Total plate counts

The results of the standard plate count are shown in Table 8. They indicate only that a relatively large number of micro-organisms are shed into the water at all times and that they persist after the swimmer leaves the tank. No attempt was made to identify any of the bacterial colonies.

Table 7. *Numbers of staphylococcus and* Staphylococcus aureus *colonies on membrane filter per* 1·0 *ml. of water sample after a* 15 *min. simulated swim period*

Subject	Condition of swimmer											
	Not menstruating				Menstruating							
					Shower				No shower			
	Shower		No shower		Tampon		No tampon		Tampon		No tampon	
	A. Sample collected immediately after the swim period											
A	61	**0**	35	**2**	96	**16**	47	**11**	—	—	130	**30**
			180	**8**								
B	34	**6**	24	**16**	50	**1**	200	**6**	—	—	80	**13**
			300	**42**								
C	42	**0**	77	**0**	48	**0**	42	**5**	58	**2**	37	**2**
D	11	**0**	78	**4**	—	—	—	—	76	**2**	120	**0**
			86	**0**							200	**0**
			23	**0**								
E	TNC	**0**	TNC	**TNC**	—	—	—	—	56	**7**	—	—
	52	**5**	350	**18**	—	—	—	—	260	**200**	—	—
	19	**5**	180	**20**								
			34	**0**								
			250	**10**								
	B. Sample collected 10 min. after the swim period											
A	48	**8**	33	**0**	48	**6**	42	**8**	—	—	140	**34**
			180	**5**								
B	13	**0**	21	**1**	51	**0**	220	**7**	—	—	79	**9**
			280	**35**								
C	43	**2**	54	**3**	35	**4**	5	**0**	60	**1**	26	**0**
D	12	**3**	120	**4**	—	—	—	—	89	**4**	91	**2**
			92	**1**	—	—	—	—	—	—	250	**15**
			18	**1**								
E	62	**5**	TNC	**TNC**	—	—	—	—	34	**6**	—	—
	42	**3**	370	**27**	—	—	—	—	230	**180**	—	—
	22	**2**	170	**10**								
			30	**7**								
			200	**5**								

The figures in bold type are counts of *Staph. aureus.*
TNC = Too numerous to count.

DISCUSSION

Since this preliminary investigation involved only a limited number of observations for each category of hygienic conditions, discussion must be limited to generalizations.

Occurrence of faecal organisms

The five participants were healthy, active young women with excellent hygienic habits; yet a marked variation in the numbers of faecal micro-organisms shed into the test water occurred under identical circumstances. Subjects B and D represent the two extremes, the former constantly shedding both coliforms and faecal streptococci under all conditions and the latter showing only minimal counts and

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then only when 100 ml. portions of water were examined by the membrane filter procedure.

The subjects showing the lowest incidence of faecal organisms were the ones who regularly used a soap containing hexachlorophene. The significance, if any, of this is not apparent at present and further investigations of this point are indicated.

Swimming while mentruating did not appear to affect the number of faecal organisms washed into the water.

Table 8. *Standard plate counts per* 1·0 *ml. of sample collected immediately after and* 10 *min. after a* 15 *min. simulated swim period*

Figures in parentheses are the counts 10 min. after the swim.

	Condition of swimmer											
					Menstruating							
	Not menstruating				Shower				No shower			
Subject	Shower		No shower		Tampon		No tampon		Tampon		No tampon	
A	14	(11)	4	(2)	93	(110)	100	(65)	0	(8)	20	(31)
	1300	(1200)	240	(240)	—		—		—	—	180	(180)
			650	(800)								
B	18	(25)	11	(18)	110	(130)	340	(600)	210	(250)	410	(330)
	270	(430)	500	(560)	—	—	—	—	590	(620)	—	—
			920	(900)								
C	45	(160)	710	(810)	32	(22)	8	(240)	14	(94)	470	(770)
	240	(70)	68	(100)	—	—	—	—	36	(46)	1500	(940)
D	65	(95)	330	(320)	750	(390)	1300	(1100)	1300	(1800)	34	(680)
			760	(440)	—	—	—	—	1800	(440)	1800	(440)
			4	(5)								
E	1000	(820)	1600	(3600)	—	—	—	—	720	(900)	—	—
	350	(350)	450	(310)	—	—	—	—	1200	(1100)	—	—
	76	(170)	290	(280)								
			20	(54)								
			560	(460)								

A comparison of the multiple tube method and the membrane filter procedure leads to the conclusion that the latter is to be preferred for the following reasons: (1) varying amounts of the sample under test may be used, thus permitting evaluation of organisms present in low concentrations; (2) results are obtained within 12–18 hr; (3) isolated colonies may be selected easily for further identification; (4) the method can be adapted, if desired, for use at the source of the sample, thus eliminating variables introduced by transport to the laboratory.

The choice between coliform organisms and faecal streptococci as indices of pollution in swimming pool waters may be evaluated on the basis of the present study. Using the multiple tube test, 19 samples were negative for both groups, 18 samples were positive for both groups, 11 samples were found to have coliforms present but no faecal streptococci, and only 2 samples were positive for the latter and negative for the former. With the membrane filter method, 9 samples were negative for both groups, 28 samples were positive for both groups, 11 samples were found to have coliforms but no faecal streptococci, and only 2 samples were positive for faecal streptococci and negative for coliforms. Thus, it is apparent that coliform bacteria represent more frequently the presence of contamination from swimmers than do the faecal streptococci.

The fact that the majority of the subjects contributed these enteric organisms to the test water lends merit to their continued use as indicators of pollution. Furthermore, the ability of these organisms to persist in the water for 10 min. after the swimmer had left the tank seems to emphasize the need for continuous residual chemical disinfection of swimming pool water.

Occurrence of body cocci

The results clearly show that the greatest number of micro-organisms shed into bathing waters by the subjects under study were cocci. Representative isolates of colonial types from the M–S agar indicated that members of the genera *Neisseria*, *Sarcina*, *Micrococcus*, and *Staphylococcus* were present. All colonies resembling *Staph. aureus* were isolated from the Staphylococcus no. 110 agar and were so classified if found to be deep yellow- or orange-pigmented, coagulase-positive, mannitol-fermenting, Gram-positive cocci. These organisms were found to be present throughout the test although they were not isolated from every water sample. However, it is clear that at all times and under all conditions of swimming, *Staph. aureus* may be shed into the water. Since generally there were lower counts of both total staphylococci and *Staph. aureus* following a shower, there is presumptive evidence, at this point, that there is some merit in the requirement for showering with warm water and soap before entering a swimming pool.

Subjects B and E, neither of whom regularly used a soap containing hexachlorophene, generally showed higher counts for these organisms than the other subjects, but it should be emphasized that this should be further explored before definite conclusions are drawn.

It is evident that body cocci are omnipresent, and clearly shown that these organisms must be considered in determining procedures for disinfecting swimming pool waters.

Streptococcus salivarius has been suggested by certain workers as an index of pollution. This organism, however, was recovered from only 28 of the 50 samples examined and thus it does not appear to be as good an index as coliform bacteria. Furthermore, this organism requires a special culture medium for isolation which, although specifically designed to encourage its growth, proved to be an excellent medium for a number of other organisms, including members of the genera *Micrococcus*, *Sarcina*, *Staphylococcus*, *Streptococcus*, *Alcaligenes*, *Bacillus*, *Lactobacillus*, and unidentified yeasts.

SUMMARY

Five healthy young women swam in untreated water of known bacterial quality under a variety of hygienic conditions. Evidence based on bacteriological examination of water samples leads to the following conclusions:

1. There is a marked variation in the number and types of bacteria shed by a

bather while swimming and the variations do not seem to be correlated to the differences in personal hygiene or the menstrual period.

2. Faecal organisms may be discharged in considerable numbers by a swimmer after a thorough and careful shower with soap and warm water and yet not be discharged in appreciable numbers by a bather who does not take a shower before swimming.

3. Faecal organisms constitute only a small minority of the total number of viable bacteria that are discharged in swimming pool water by a bather during the act of swimming and as such seem to have limited use as indicators of *total* bacterial pollution.

4. Members of the genus *Staphylococcus* are shed in large numbers under all conditions and *Staph. aureus* is consistently present. Therefore, this genus appears to be a good choice as an index for the determination of body contamination.

5. Further studies are indicated under more stringently controlled hygienic conditions to determine the value of hexachlorophene in reducing microbial flora that a given individual may shed during swimming.

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