

Induction of Inflammation, Fever, and Acceleration of Adjuvant-Disease by Surfactants (33500)

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Arthritis has been produced in laboratory animals by the intraarticular inoculation of streptolysin S and filimarisin (Filipin) (1, 2). These drugs, along with other surfactants, produce lysis of various cellular membranes (3, 4). Many of these surfactants combine with cholesterol and this phenomenon is

TABLE I. Hindpaw Edema Produced by Local Inoculation of Various Surfactants.^a

Drug	Saline ($\mu\text{g}/0.1$ ml) into right paw	Hindpaw edema (mg/100 g of BW)
Filipin	5	63 \pm 7
	10	80 \pm 9
	20	146 \pm 13
	40	226 \pm 32
	80	305 \pm 21
	160	382 \pm 34
	320	496 \pm 27
	640	539 \pm 25
Saponin	5	85 \pm 10
	10	112 \pm 2
	20	197 \pm 8
	40	294 \pm 15
	80	373 \pm 18
	160	488 \pm 34
	320	570 \pm 31
Digitonin	25	261 \pm 7
	50	261 \pm 14
	100	324 \pm 11
	200	370 \pm 11
	400	380 \pm 12
Nystatin	500	189 \pm 12
	500	567 \pm 17
Etruscomycin	500	101 \pm 13
Cholesterol	8000	0
Vehiele (NaCl)	0.1 ml	0

^a Ten rats/group; fasted 4 p.m.–8 a.m.; injection into paws as shown at 8 a.m. and paws were removed 2 p.m. or 6 hr later; \pm = SEM in this and in all other tables; BW = Body weight in this and all other tables.

TABLE II. Persistence of Inflammation Following Local Inoculation of Filipin, Saponin, and Digitonin.^a

After inj. (hr)	Edema (mg/100 g of BW)		
	Filipin	Saponin	Digitonin
0	0	0	0
1	273 \pm 26	—	—
2	451 \pm 13	—	—
4	530 \pm 16	502 \pm 20	780 \pm 15
6	—	680 \pm 12	910 \pm 10
8	527 \pm 17	—	—
16	444 \pm 35	—	—
24	498 \pm 22	390 \pm 20	550 \pm 7
48	321 \pm 22	270 \pm 11	400 \pm 6
72	304 \pm 18	220 \pm 10	—
96	290 \pm 15	210 \pm 9	280 \pm 10
144	—	110 \pm 14	—

^a Five rats/group; filipin, saponin, and digitonin (1.0 mg/0.1 ml of saline) were inoculated into right hindpaw at 0 time; paws were removed and difference between injected and uninjected paws was recorded as shown.

thought to be responsible for their lytic effects (5–7). The following studies were done to determine the inflammatory–pyrogenic–arthritogenic effects of digitonin, Filipin, and saponin.

Methods. Male Sprague-Dawley rats (170–220 g) were used throughout. Digitonin, Filipin, and saponin (a mixture from Bell-Mathieson-Coleman) were injected as shown in the various tables.

Hindpaw inflammation was produced by injecting various agents into the right hindpaw in 0.1 ml of sterile saline. The injected and uninjected paws were severed with a Harvard guillotine at various times thereafter and weighed. The differences were expressed as edema/100 g of body weight. Variations of this procedure have been described (8).

Fevers were recorded by means of intra-peritoneally-placed thermistor probes at the indicated times.

TABLE III. Effects of Varying Amounts of Carrageenin and Digitonin on Inflammatory Edema of Hindpaw of Rats.^a

Compound	Right paw (μg)	BW (g)		Edema (g/100 g of BW)		Potency
		Mean	SD	Mean	SD	
No treatment	0	242	4	-0.017	0.003	
Carrageenin	25	238	4	0.053	0.005	1.0
	50	231	3	0.050	0.008	
	100	231	2	0.121	0.009	
	200	233	7	0.141	0.010	
	400	229	6	0.226	0.016	
	800	229	4	0.301	0.009	
Digitonin	25	229	2	0.261	0.007	20.6
	50	235	3	0.261	0.014	(15-28)
	100	222	6	0.324	0.011	95%
	200	234	4	0.370	0.011	
	400	225	6	0.380	0.010	
	800	236	6	0.428	0.012	

^a Right hindpaws were injected at 0 time and paws were removed 6 hr later; 10 rats/group; SD = standard deviation in this and all other tables where used.

Adjuvant-induced polyarthritis (9-15) was produced by the inoculation of 0.5 mg of *M. butyricum* in 0.1 ml of heavy mineral oil into the tail. The advent of clinically detectable disease was recorded daily.

Anti-inflammatory drugs were given orally as suspensions in water (5.0 ml/kg).

Results. Digitonin, Filipin, and saponin produce dose-related inflammatory effects when injected into the hindpaw of rats (Table I). The polyene antibiotic, etruscomycin, but not nystatin and cholesterol, also pro-

duces significant inflammation when injected into the hindpaws of rats. The maximal inflammatory effect is produced within 4-8 hr and persists for at least 144 hr (Table II). When digitonin is reinjected at 144 hrs, the same time course is observed; another inflammatory process reaches its maximum in 4-8 hr and subsides within 144 hr, 288 hr after the first injection. Unlike results with *M. butyricum* and *M. tuberculosis*, the intradermal injection of digitonin; followed by its intrapaw injection 2 weeks later, does not

TABLE IV. Inhibition of Hindpaw Edema Induced by Digitonin and Carrageenin.^a

	Dose (mg/kg)	Edema (g/100 g of BW)		SD		Percentage inhibition	
		Carrageenin	Digitonin	C	D	C	D
		(C)	(D)				
Water vehicle	—	0.267	0.284	0.013	0.010	0	0
Phenylbutazone	12	0.235	0.212	0.016	0.010	12	25
	36	0.206	0.194	0.012	0.022	23	32
	115	0.207	0.158	0.006	0.008	22	44
Indomethacin	1.2	0.204	0.207	0.011	0.008	23	27
	3.6	0.183	0.178	0.011	0.008	31	37
	12.0	0.174	0.149	0.010	0.014	35	47

^a Ten rats/group; injection into right hindpaws of 50 μg of carrageenin and 50 μg digitonin in 0.1 ml of saline 1 hr after oral administration of drugs; paws were removed 6 hr after hindpaw injections or 7 hr after drugs.

TABLE V. Oral Potencies of Various Drugs on Carrageenin and Digitonin-Induced Inflammation.^a

Compound	Dose (mg/kg)	Edema (g/100 g of BW)		SD		Drug potency ratio	
		Carrageenin (C)	Digitonin (D)	C	D	C	D
Water vehicle	—	0.293	0.341	0.013	0.012	0	0
Phenylbutazone	4	0.277	0.311	0.016	0.012		
	12	0.243	0.268	0.010	0.006		
	36	0.221	0.251	0.015	0.013	1.0	1.0
	108	0.205	0.211	0.009	0.012		
Indomethacin	0.4	0.285	0.282	0.012	0.011		
	1.2	0.230	0.304	0.012	0.013		
	4.0	0.172	0.235	0.011	0.017	15.2	8.8
	12.0	0.182	0.226	0.010	0.010	(8-30) ^b	(5-16)
Aspirin	13.0	0.227	0.283	0.032	0.011		
	40.0	0.253	0.277	0.030	0.013		
	120.0	0.203	0.227	0.012	0.008	0.95	0.92
	352.0	0.099	0.124	0.020	0.011	(0.5-1.9)	(0.5-1.8)
6-Alpha-methyl-prednisolone	0.4	0.277	0.294	0.018	0.018		
	1.4	0.214	0.248	0.021	0.014		
	4.0	0.139	0.239	0.011	0.014	67.8	13.2
	12.0	0.072	0.217	0.007	0.010	(34-148)	(7.4-24.0)

^a Ten rats/group; carrageenin (500 μg) and digitonin (100 μg) were injected into right hindpaws 1 hr after oral administration of drugs; paws were removed 7 hr after drug or 6 hr after paw injections.

^b (8-30) is the 95% confidence limit.

result in a "delayed-type" hypersensitivity reaction.

Digitonin is at least 20 times more potent than carrageenin toward the production of hindpaw edema in rats (Table III). Phenylbutazone and indomethacin inhibit both carrageenin and digitonin induced hindpaw edema (Table IV). The oral potency estimates for indomethacin and 6-alpha-methylprednisolone (Medrol) are lower when digitonin is used as the phylogistin (Table V). Aspirin is equipotent in both assays.

Digitonin, Filipin, saponin, and yeast produce dose-related fevers (Table VI); fevers persist from 16-24 hr. When injected every 24 hr, the digitonin-induced fevers persist throughout the injection interval (Table VII). Digitonin-induced fever occurs more rapidly than that produced by Filipin and saponin (Table VIII). Like the inflammatory process, the fever produced by digitonin is inhibited also by phenylbutazone and indomethacin (Table IX).

When incorporated in *M. butyricum*: min-

eral oil suspensions as a 2% mixture, digitonin causes arthritis to appear 5-7 days earlier. The joint disease is also more severe than that produced by injection of *M. butyricum* in mineral oil alone (Table X). *M. butyricum* fails to produce arthritis when given with and without digitonin in saline. Filipin and saponin combined with mineral oil-*M. butyricum* also induce arthritis in rats within 10-12 days.

Summary and Discussion. Saponin, Filipin, and digitonin produce acute inflammation and fever when injected into the hindpaw or subcutaneously. Digitonin-induced inflammation and fever are inhibited by anti-inflammatory drugs. Digitonin is at least 20 times more potent than carrageenin on a weight basis when injected into the hindpaw of rats.

Digitonin, Filipin, and saponin, when incorporated into the mineral oil-*M. butyricum* mixture, causes rats to develop arthritis more rapidly. The destruction of *M. butyricum* by granulocytes may be one of the limit-

TABLE VI. Production of Fever by Various Surfactants and by Yeast.^a

Compound	Dose (mg/rat, s.c.)	Postinjection (hr):	Body temperature by intraperitoneal thermistor probe	
			16	24
Vehicle (NaCl)	—		98.7 ± 0.2	98.2 ± 0.1
Digitonin	1		98.3 ± 0.1	98.1 ± 0.2
	2		99.4 ± 0.1	98.5 ± 0.2
	4		99.4 ± 0.1	98.5 ± 0.1
	8		99.9 ± 0.2	98.8 ± 0.3
	16		100.9 ± 0.2	100.4 ± 0.2
	32		100.6 ± 0.1	100.4 ± 0.3
Filipin	1		99.4 ± 0.1	99.0 ± 0.3
	2		100.2 ± 0.3	100.0 ± 0.2
	4		100.0 ± 0.3	99.1 ± 0.2
	8		100.0 ± 0.2	99.6 ± 0.1
	16		100.5 ± 0.3	99.4 ± 0.3
	32		99.6 ± 0.4	99.0 ± 0.4
Saponin	1		100.2 ± 0.2	100.1 ± 0.2
	2		100.5 ± 0.2	99.0 ± 0.4
	4		100.0 ± 0.1	98.4 ± 0.8
	8		96.2 ± 0.5	95.5 ± 0.6
	16		92.8 ± 0.9	89.8 ± 0.9
	32		87.2 ± 0.9	84.5 ± 1.0
Yeast (Bakers)	50		99.0 ± 0.1	—
	100		99.2 ± 0.09	—
	200		99.7 ± 0.1	—
	400		100.4 ± 0.09	—
	800		100.4 ± 0.2	—

^a Five rats/group; drugs were given subcutaneously at 4 p.m.; fevers were measured at 8 a.m. following day and, in cases shown, again at 2 p.m.; rats on three highest doses of saponin appeared moribund.

TABLE VII. Effect of Repeated Injections of Digitonin on the Induction of Fever in the Rat.^a

No. of days injected (16.0 mg of digitonin, s.c.)	Days:	Body temp (°F)						
		1	2	3	4	5	6	7
Vehicle (0.5 ml, s.c.; days 0-4)		99.1	99.1	99.0	99.3	98.5	98.9	98.3
	±:	0.1	0.2	0.1	0.1	0.2	0.1	0.2
Day 0		100.1	99.4	99.1	99.3	98.5	98.9	98.6
	±:	0.2	0.1	0.2	0.2	0.1	0.3	0.2
Day 0 + 1		100.2	100.4	99.4	99.5	98.9	99.4	99.0
	±:	0.1	0.3	0.2	0.1	0.2	0.2	0.1
Day 0 + 1 + 2		100.8	100.3	100.0	99.6	99.1	99.8	99.1
	±:	0.1	0.1	0.1	0.04	0.1	0.1	0.1
Day 0 + 1 + 2 + 3		100.6	100.1	100.0	100.3	99.5	99.7	99.4 ^b
	±:	0.1	0.1	0.3	0.2	0.3	0.2	0.2

^a Ten rats/group; injections as shown at 8 a.m. every 24 hr; fevers were recorded 30-60 min prior to digitonin injections on respective days.

^b Animals dead (2/10) after repeated injections for 4 days and 5/10 obviously sick.

TABLE VIII. Time Course of Fever Production in Response to Filipin, Digitonin, and Saponin.*

Drug	Dose (mg, s.c.; time 0)	(hr):	Body temp (°F)						
			2	4	6	8	16	24	48
NaCl	1.0 (ml)		97.4	98.2	98.0	98.1	99.1	98.2	98.3
		±:	0.1	0.1	0.1	0.2	0.2	0.1	0.1
Filipin	16.0		97.5	98.6	98.5	98.9	<i>100.2</i>	99.3	98.4
		±:	0.1	0.2	0.3	0.2	0.1	0.1	0.1
Digitonin	16.0		98.0	<i>100.2</i>	<i>101.4</i>	<i>101.7</i>	<i>101.2</i>	<i>100.7</i>	98.4
		±:	0.2	0.3	0.2	0.1	0.1	0.3	0.2
Saponin	3.0		97.9	98.7	<i>99.4</i>	<i>99.5</i>	<i>100.1</i>	98.8	98.4
		±:	0.2	0.1	0.2	0.1	0.1	0.2	0.1

* Ten rats/group; drugs injected s.c. in 1.0 ml of physiological saline; italicized numbers indicate times at which temperatures were above normal.

TABLE IX. Effect of Standard Anti-inflammatory Drugs on Digitonin-Induced Fever.*

Drug	Dose (mg/kg; p.o.)	Change (°F)	Inhibition (%)
1. No treatment	—	—	—
2. Digitonin	—	+3.0	—
2 + Phenylbutazone	13	+2.9	0
	39	+1.8	40
	116	+0.4	87
2 + Indomethacin	1	+2.8	0
	4	+1.7	44
	13	+1.2	60

* Ten rats/group; digitonin (16.0 mg) was injected s.c. 1 hr after oral administration of drug; fevers were recorded 6 hr after drug; normal temperature is $97.8 \pm 0.08^\circ\text{F}$.

ing factors in its ability to produce disease. The bacterium does not produce arthritis when inoculated in saline. Like mineral oil, digitonin and other surfactants may prolong the survival time of *M. butyricum in vivo*. Lytic agents like digitonin may be added to the list of other available "adjuvants" for the acceleration of immunologic phenomena (16-18).

Filipin and Medrol were obtained from The Upjohn Company, indomethacin and phenylbutazone from Merck, Sharp and Dohme and Geigy Pharmaceuticals, respectively.

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TABLE X. Effect of Digitonin on the Onset of Adjuvant-Induced Polyarthritis in Rats.*

Treatment	Day on which arthritis became visible and no. of rats with disease								
	12	13	14	15	16	17	18	19	
Mineral oil (0.1 ml)	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
<i>M. butyricum</i> (0.5 mg) : mineral oil (0.1 ml)	0/20	0/20	0/20	3/20	7/20	8/20	10/20	11/20	
<i>M. butyricum</i> (0.5 mg) : mineral oil (0.1 ml) digitonin (2%)	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20

* All injections were into tail as shown; rats with digitonin-*M. butyricum*-induced polyarthritis had more severe disease than those injected with bacterium: mineral oil, alone.

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"Induction" of Viral Antigen in Established Cell Line (SP-8) Derived from Shope Virus-induced Cutaneous Papilloma of Rabbits* (33501)

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In a previous report from this laboratory, the establishment of a cell line (SP-8) from Shope papilloma virus (SPV)-induced papillomas of rabbit skin was described (1). The presence of SPV antigen was clearly demonstrated as the specific nuclear immunofluorescence in the primary culture of the SP-8 cells (2). However, the number of fluorescent cells decreased at each subcultivation level and eventually disappeared completely at the fourth transfer generation. Since then, fluorescent cells have not been observed to date. The present communication describes findings on the attempts to "induce" the synthesis of SPV antigen in these nonreacting

SP-8 cells by lowering the temperature of incubation from 37 to 30°

Materials and methods. The SP-8 cells successively passaged for over 80 generations at 37° as a stationary culture were used for the present experiment. The SP-8 cells were grown in monolayer cultures employing YLE medium (Earle's balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate) plus 20% calf serum and 10% tryptose phosphate broth as growth medium. Maintenance medium was composed of YLE medium plus 2% calf serum. For the immunofluorescent studies, SP-8 cells were grown for 3 days on a coverslip in Leighton tubes. After the monolayer was formed, they were washed three times with maintenance medium. Subsequently, the maintenance medium was added to the washed cell cultures and the cultivation was resumed either at 37° or at 30° for the duration of the experiment.

The indirect staining method was used for the immunofluorescent studies (1, 2). Cov-

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