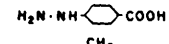
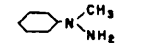
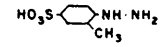
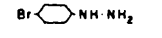
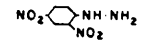
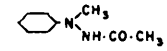
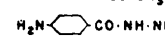
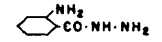
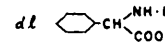
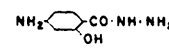
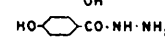
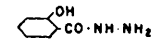
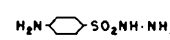
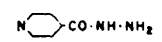
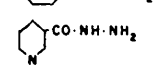
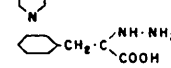
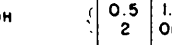
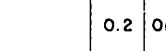
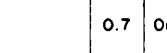
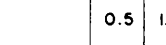
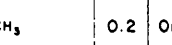
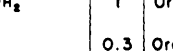
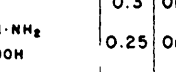
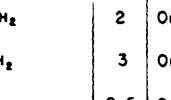
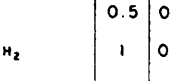
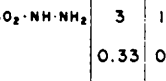
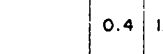
(Table I continued)

No.	Formula	Dosage mg. per animal	Route	No. of treat. per wk.	Increase of life over contr. in days
35		0.5 2	I.P. Oral	3 3	0 0
36		0.2	Oral	6	1
37		3	I.P.	5	0
38		0.7	Oral	3	0
39		0.5	I.P.	5	0
40		0.2	Oral	5	0
41		1	I.P.	3	0
42		1	Oral	3	-1
43		0.3	Oral	5	-2
44	*dl* 	0.25	Oral	3	-1
45		2	Oral	6	-1
46		3	Oral	6	-2
47		0.5	Oral	6	-2
48		1	Oral	6	0
49		3	I.P.	3	0
50		0.33	Oral	6	-2
51		0.4	I.P.	6	0
52		2	Oral	6	-1
53		0.4	Oral	5	0
54		1	Oral	3	0
55		0.17	Oral	3	0
56		0.5	Oral	3	1
57		6	Oral	6	-1
58		0.07	Oral	5	1
59		1	I.P.	5	0
60		0.5	I.P.	3	0
61		4	I.P.	3	1
62		12	I.P.	6	0
63		2	Oral	6	1
64		1	I.P.	6	1
65		2	I.P.	5	0
66		4	Oral	6	-2
*	Urethane	8	I.P.	3	2.4
*	Potassium Arsenite	0.1	I.P.	3	0

* Both Urethane and Potassium Arsenite were included for comparison.

The compounds were prepared in this laboratory with the following exceptions:- Nos. 3, 10, 29, 36, 38 were obtained from Distillation Products Corp., No. 37 from General Dyestuff Corp., No. 65 from the Dow Chemical Co., and No. 66 from the Geigy Chemical Co.

pounds. None was found active.

1. Boyland, E., *Biochem. J.*, 1938, v32, 1207.
2. ———, *Biochem. J.*, 1946, v40, 55.
3. ———, *Biochem. J.*, 1941, v35, 1283.
4. Burchenal, J. H., Lester, R. A., Riley, J. B., and Rhoads, C. P., *Cancer*, 1948, v1, 399.
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Sugar Alcohols. XXIX. Sorbitan Polyoxyethylene Fatty Acid Esters and Polyoxyethylene Monostearate and Respiration of Kidney. (19970)

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For many years our studies(1) have been designed to elucidate the fate of the sugar alcohols and their derivatives(2) in the animal body. The widespread use of the derivatives of sorbitol as non-ionic emulsifiers in such common components of the human diet as ice cream, bread and other bakery products has focused attention upon the effects of these

TABLE I. Effect of Emulsifying Agents on Oxygen Uptake of Kidney.

Control Qo_2	No. determinations		Qo_2	No. determinations	% change	P value
11.9 S.E. .32	23	Tween 80, 1%	11 S.E. .53	11	—7.6	>.1
14.5 " .38	8	Tween 20, 1%	16 " .66	8	+9.4	>.05
14.1 " .46	8	Tween 60, 1%	12.8 " .79	8	—9.7	>.05
13.5 " .44	10	Tween 81, 1%	13.8 " .38	11	+2	>.1
15.2 " .80	8	Myrj 52, 1%	15.3 " .60	8	+ .50	>.9

agents upon various structures of the body. Schweingert, McBride, and Carlson(3) fed two polyoxyethylene monostearate products to weanling hamsters at levels of 5 to 10% and observed that their presence in the diet produced a retarded growth rate and other deleterious effects, such as marked diarrhea and changes in the gastrointestinal tract. On the other hand, extensive feeding experiments (2) in this laboratory using rats, monkeys, dogs and mice failed to reveal these effects. Schulz, Madaus and Soehring(4) studied the effect of certain surface active agents similar to Tweens 20 and 80 on the oxygen uptake of yeast cells and *E. coli*. In general, they observed that low concentrations enhanced gaseous metabolism and higher levels impeded it. They attributed the effect to surface activity and correlated it with the molecular weight of the surface active agents.

Owing to the interest in tissue response to these emulsifiers we decided to study the effect of these agents upon the respiration of kidney tissue.

Substances used. The substances used for this study were:

Tween* 80—
polyoxyethylene (20) sorbitan monooleate

Tween 20—
polyoxyethylene " " "

Tween 60—
polyoxyethylene " " monostearate

Tween 81—
polyoxyethylene (5) " monooleate

Myrj 52—
polyoxyethylene (40) stearic acid

Experimental. Male rats weighing between 300 and 450 g were killed by a blow on the head. The kidneys were removed and sliced. The oxygen uptake of approximately 100 mg of kidney tissue was determined by the standard Warburg technic. The substrate was a modified Krebs-Ringer's solution with 0.2% glucose. Three cc of Ringer's solution was used for the controls, and 0.3 cc of a 1% solution of the various emulsifying agents was added to 2.7 cc Ringer's solution for the experimental runs.

After an equilibration period of 10 min the emulsifying agents were added to the tissue and readings were taken every 15 min for 1 hr. The Qo_2 for 1 hr for both experimental and control runs were calculated and compared. The results are shown in Table I.

None of the changes in Qo_2 produced by the various emulsifying agents are considered significant.

Discussion. It has been well established that the ionic surface active agents such as benzalkonium chloride, materially interfere with the cellular metabolism of bacteria. Dubos(5) showed in 1946 that trace quantities of Tween 80 permitted submerged and rapid growth of the tubercular bacillus *in vitro*. On the other hand, the presence of small quantities of Tween 20 (0.005%) depressed the metabolism of the organism. Since the emulsifiers of the Myrj and Tween types are being extensively used in foods, and hence coming in contact with the tissues of the body, it is of interest to note that in concentration of 0.1 of 1% these substances do not interfere with the oxidation of glucose in kidney tissues.

Summary. In kidney slices the addition of Myrj 52, and Tweens 20, 60, 80 and 81, do not significantly interfere with the oxygen uptake of kidney tissue as determined by the

* These are trade names employed for these substances as manufactured by the Atlas Powder Co. of Wilmington, Del.

Warburg technic.

1. Carr, C. J., and Krantz, John C., Jr., *Advances in Carbohydrate Chemistry*, I, 1945, Academic Press, Inc., New York. (Metabolism of Sugar Alcohols).
2. Krantz, John C., Jr., Culver, Perry, Carr, C. J., and Jones, Chester, *Bull. School of Med.*, Univ. of Maryland, 1951, v36, 48.
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Virus Growth in Tissue Culture Fibroblasts. II. Coxsackie Virus (Group B) in Cultures of Mouse Fat Tissue. (19971)

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(Introduced by Icie G. Macy.)
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One of the distinguishing features of the B group of Coxsackie viruses is their ability to produce histopathologic changes in adipose tissues of infant mice(1-3). Although virus has been recovered from these tissues, this does not constitute proof that the virus has specifically infected the cells found in embryonal fat tissues(2,3). Current tissue culture technics offer an opportunity to demonstrate: a) whether a group B Coxsackie virus can be propagated in adipose tissues *in vitro* and b) whether there is a viral susceptibility relationship between the cells of the host tissue and fibroblasts derived from it. In this demonstration we have determined the growth patterns and cytopathogenic effects of a group B type 1 Coxsackie virus in tissue cultures of interscapular fat pads of newborn mice as well as in cultures of fibroblasts derived from this tissue.

Materials and methods. Tissue cultures. A modification of the Porter roller flask method(4) utilizing clotted plasma was employed. The nutrient fluid consisted of 10% chick embryo extract and 30% horse serum incorporated in Hank's balanced salt solution. The initial pH of the nutrient medium was adjusted to 7.4-7.6 with 1.4% (isotonic) $NaHCO_3$. Phenol red in a final concentration of 0.002% was used as indicator. To the medium were added 100 units of penicillin and 100 micrograms of streptomycin per milliliter to control bacterial contamination. Two drops of chicken plasma were placed on cover slips cut to provide for insertion easily into a Porter flask. With a curved-tip pipette 2 tissue explants were taken up in 2 drops of nutrient fluid and added to the plasma on each cover slip. Following coagulation of the plasma, 2 cover slips, each containing 2 embedded tissue fragments, were each placed on the opposite flat surfaces of a Porter flask. They adhered firmly after the flat inner surfaces of the flasks were wet with nutrient fluid. After adding 1 ml of nutrient medium the flasks were tightly stoppered, placed in a rotating drum (⅕ rpm) modified to hold the Porter flasks, and incubated at 36°C. For routine maintenance of the cultures, 3 times each week the nutrient fluid was replaced and lysis of the plasma clot was repaired with fresh plasma. To divide cell colonies the cover slips were removed from the flasks and the fibroblastic outgrowths dissected into portions approximately 1.0 x 1.0 mm for replantation. *Source of cultured cells.* One-half millimeter fragments were prepared from the interscapular fat pads of newborn mice. Four explants and their resulting cellular outgrowths were designated primary culture passages. When colonies reached maximum size they were dissected and portions were transferred to other flasks for the second and third passages. After they had attained their maximum sizes colonies of the primary culture passages contained a central area consisting of tissue carried over from the animal host and a surrounding 2-3 mm band of outgrowth consisting of fibro-