

- (32) Burn, "Methods of Biological Assay," Oxford Univ. Press, 16 (1928).
 (33) Report of the Committee on Pharmacology and Bioassays of the A. PH. A., July 29, 1931.
 (34) Bauer and Fromherz, *Arch. exptl. Path. Pharmacol.*, 172, 693 (1933).
 (35) Burn, "Methods of Biological Assay," Oxford Univ. Press, 117 (1928).
 (36) Biilmann, *Bull. soc. chim. France*, 41, 213 (1927).
 (37) Cullen and Biilmann, *J. Biol. Chem.*, 64, 727 (1925).
 (38) Smith, *Jour. A. PH. A.*, 17, 241 (1928).
 (39) Haag and Jarrett, *Ibid.*, 20, 471 (1931).
 (40) Wokes, *Quart. J. Pharm. Pharmacol.*, 3, 205 (1930).
 (41) Foster and Van Dyke, *Jour. A. PH. A.*, 22, 381 (1933).
 (42) Circular obtained by private communication with Dr. C. A. Morrell and Dr. C. W. Chapman.
 (43) British Pharmacopoeia, 621 (1932).
 (44) *A. D. M. A. Proc.*, 302 (1934).
 (45) *Ibid.*, 292 (1935).
 (46) Berry and Davis, *Quart. J. Pharm. Pharmacol.*, 8, 443 (1935).

STUDIES OF LECITHIN SOAP.*

I. BACTERICIDAL AND DETOXIFYING EFFECT ON INTESTINAL FLORA.

BY L. G. HADJOPOULOS AND SAUL CASPE.

The detergent properties of soaps have been known since their introduction into domestic use. A definite knowledge of their bactericidal value was gradually acquired at a much later period through the observations of such outstanding workers as Koch (1881), Noguchi (1907), Landsteiner and Erlich (1908), Lanar and Flexner (1911), Nichols (1920) and others.

During the last two decades, the bacteriostatic property of soaps has been intensively investigated and the results obtained can be summarized in the words of Eggerth (1-5):

1. That there exists within certain limitations, a direct relationship between the molecular weights of the fatty acids, and the corresponding germicidal properties of their respective soaps;
2. That the germicidal properties are more manifest in the higher p_H values of the soaps;
3. That the soaps of the various fatty acids exhibit selective germicidal values which can be increased or decreased by substituting the alpha hydrogen with hydroxyl, bromine or sulphydryl-groups.

R. R. Spencer (6) experimentally confirmed the selectivity of certain soaps for certain bacteria, and his interesting work on the visible effects of sodium ricinoleate in completely dissolving bacterium tularense, bacillus typhosus, bacillus pyocaneus, bacillus alkaligenes, can be used as a working hypothesis of the mode by which soaps act upon bacteria.

Larson (7) assumed that, in general, these intrinsic properties of soaps are dependent upon their surface tension reducing values. However, he was not able to account by this assumption for the effectiveness of soaps upon bacterial by-products

* Presented before Scientific Section, A. PH. A., New York meeting, 1937.

¹ Consultive Chemist, 40 N. 86th St., N. Y. C.

of the nature of exotoxins, and therefore postulated that this latter activity may be referred to the difference between the electric charge of the soap and toxin molecule.

Based on these observations, Larson suggests that

“The biliary soaps undoubtedly play an important rôle in detoxifying the bacterial toxins of the intestinal tract. The intestinal tract is essentially a thirty-foot culture tube which harbors a large variety of bacteria, many of which growing under favorable conditions, undoubtedly secrete toxins. Had nature not provided an efficient detoxifying mechanism in the form of bile soaps, the host probably would not have survived.”

The therapeutic management of intestinal antiseptics and detoxification by use of soaps is predicated upon these principles.

In 1928, we repeated some of the work reported by Eggerth and Larson, and noted a limitation to the general use of soaps as intestinal antiseptics: soaps are irritating to the gastrointestinal mucosa. In an attempt to overcome this disadvantage, we reduced the high alkalinity of the soaps to almost the neutral point, only to witness the fact already noted by Eggerth that these neutral soaps were of diminished therapeutic value.

By administering soaps in the form of pills coated with salol or protected by keratine, we might avoid the undue irritation to the gastric mucosa, especially in cases of hypoacidity and achlorhydria; but our experience with pills was highly unsatisfactory as such a compact mass of soap would invariably escape the intestinal tract without a sufficient amount of emulsification.

Our problem was, therefore, to derive a soap which would pass the gastric mucosa untouched but would readily emulsify in the intestines with the least possible irritation to both. In other words, a soap equally or sufficiently potent at a low p_{H} , easily taken by mouth in the form of a finely triturated powder and readily emulsified in the intestines.

In the quest of a product with the above qualifications, we soon came to the conclusion that we had to limit our sources to a small group of fatty acids as the most readily emulsifiable bases for soaps. The soaps of the fat-like substances derived from tissue extracts (lipids, lecithids) were found to answer the above qualifications.

The bactericidal selectivity displayed by the ordinary fatty-acid soaps was also noticed in the normal sodium salts of such extractives. Thus, heart tissue extracts (lipids) were more active against the gram-positive group of cocci (even *Streptococcus Fecalis*) while the liver and kidney extracts were more active against the gram-negative paracolony group of bacilli.

In Table I we give in a condensed form a list of experiments to demonstrate the superiority of lipin to ordinary soaps in their relative immediate and remote effects on intestinal flora.

After having chosen as our control a man with no acute or chronic symptoms referable to the gastro-intestinal tract, except a moderate degree of constipation, we made repeated bacteriological examinations of his stools to determine his basal intestinal flora. The results of his final stool examination on 1/15/29, which was in no way different from the previous findings, is given in the first line of Table I.

TABLE I.—THE COMPARATIVE BACTERICIDAL PROPERTIES OF VARIOUS SOAPS ON THE INTESTINAL FLORA.

Date, Medication:	Type of Soap.	Stools Examined.	<i>Streptococcus</i> . Hemol.	Virid.	Colon Group Bacillus.	Effect on the G. I. Mucosa.
Control	No medication	1/14/29	0	Innumerable	Innumerable	Normal
1/15/29	Na Lecithid	1/16/29	0	0	0	No unpleasant effect
		1/17/29	0	0	0
		1/18/29	0	0	0
		1/19/29	0	0	0
		1/20/29	0	0	Innumerable
2/6/29	Ord. Comm. Soap	1/21/29	0	500	Innumerable
		2/7/29	1	10	Innumerable	Slight irritation
		2/8/29	0	1	Innumerable	Rather constipating
		2/9/29	0	2	0
		2/12/29	0	11	Innumerable
2/17/29	Na Ricinoleate	2/18/29	0	5	15	Markedly irritating
		2/19/29	0	0	50
2/20/29	Na Ricinoleate	2/21/29	0	3	15
2/22/29	Na Ricinoleate	2/23/29	0	1	400
3/19/29	Na Stearate	3/20/29	0	4	20	Slightly irritating
		3/21/29	0	37	4	Rather constipating
		3/22/29	0	500	Innumerable
		6/17/29	Na Oleate	6/18/29	15	100
2/5/30	Na Palmitate	6/19/29	21	5	200
		6/20/29	0	30	3
		2/6/30	0	1	Innumerable	Fairly well borne
		2/7/30	0	50	Innumerable
		2/8/30	0	450	Innumerable

COMMENTS: The results, as tabulated, were the result of a single 5-grain dose of the particular soap administered by mouth. We made an exception to this rule in the case of Sod. Ricinoleate for reasons to be mentioned later.

He then submitted to the various soap treatments at sufficient time intervals so as to eliminate the possibility of the effects of one treatment running over to the other. The bacteriological column in Table I was divided into *Streptococcus* (98% *Str. Fecalis*) and *B. Coli*. The *Streptococci* were further differentiated into Hemolytic and Viridans or Anhemolytic. We made no further attempt to differentiate the *Coli*-bacillus group. The number of bacteria was determined from pour blood plate colonizations: a small loopful of stools was thoroughly emulsified in one cc. of neutral broth and mixed in ten cc. of nutrient agar with human blood from which the plates were poured.

Of all the soaps tested the best borne and the most effective clinically was the Lecithid product. Its effect was immediate and uniform, keeping the stools sterile for four days. On the fifth day *B. Coli* reappeared and on the sixth the *Streptococci*. Of the other soaps, the palmitate, the next best borne by mouth, was but weakly active on *Streptococci* and completely inactive for *B. Coli*. The stearate showed a fair degree of activity on both flora, but its effect was of shorter duration. Besides, it manifested a moderate degree of intestinal irritation and tendency toward constipation. Sodium Oleate, the unsaturated fatty acid soap, was probably the most satisfactory of the pure fatty acid group of soaps, although it could not sterilize

completely the stools even for one day. The appearance of hemolytic *Streptococci* during its administration, a fact for which we have no explanation, was surely an undesirable complication. In the case of commercial soaps, the source of which is generally animal and vegetable fats of a mixed fatty acid composition, the results were within ordinary expectations. Finally, Sod. Ricinoleate displayed a fair degree of activity on both intestinal flora, but it was markedly irritating to gastrointestinal mucosa. In an attempt to fortify and prolong its action, which as one will notice in our table, is not very strong, we administered it every other day to a total of 15 grains. The results, however, were not any different from those of the first dose.

Having thus convinced ourselves about the superiority of Lecithid soaps to others we next endeavored to test its effectiveness in chronic intestinal disorders of

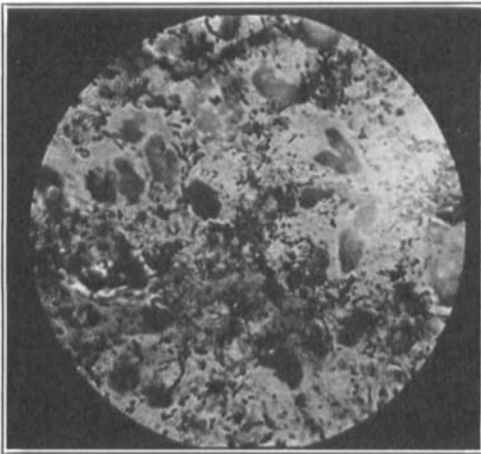


Fig. 1 shows the condition of stools a day before Na Lecithid was administered. Note the abundance of mixed bacterial flora, especially the diplococci and streptococci chains. The rest of the fecal material is made up mostly of mucous plugs, erythrocytes and a few degenerated and swollen epithelial cells.

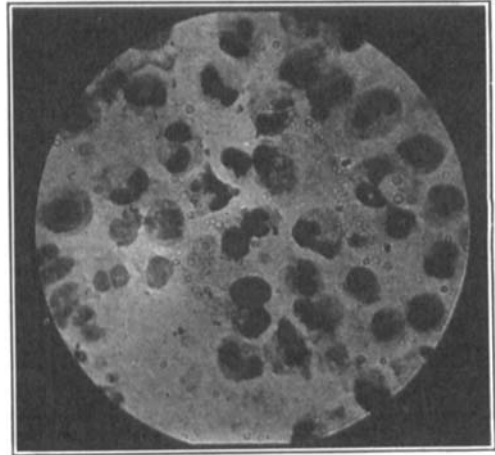


Fig. 2.—A day after medication the stool takes a different aspect. The number of bacteria is reduced to an occasional diplococcus or chain mostly engulfed within phagocytic leucocytes and epithelial cells. Mucous plugs are practically absent and erythrocytes less in number.

the general type where *B. Acidophilis* implantations are clinically recommended. As such cases, we then had at our command a long list of chronic sufferers from Rheumatoid and Hypertropic Arthritis. We instituted this treatment in over a hundred (now over a thousand) cases administering once a week 2 grains of Na Lecithid triturated in 5 grains of Lactose and 1 grain of Phenolphthalein. Clinically our results on the whole were very satisfactory but bacteriologically we were somewhat disappointed in finding out that a quick sterilization of the stools, which was so readily accomplished in normal individuals, was still a problem in these chronic sufferers. It usually took weeks and often months before we could witness a sterile stool in our patients.

To avoid discrimination and bias in selecting cases for report, we tabulated them alphabetically and in Table II we give the first 15 of a list of 100.

TABLE II.—THE EFFECT OF NA LECITHID ON INTESTINAL STREPTOCOCCI IN ARTHRITICS.

Serial Number.	Name Abr.	Treatment Instituted.	Number Colonies per Plate.	Date Bact. Improvement.	Number Colonies per Plate.
1	App.	4/23/30	Innumerable	10/17/30	1
2	Aus.	4/29/30	Innumerable	9/23/30	20
3	Bru.	8/5/30	Innumerable	11/4/30	0
4	Boy.	5/21/30	Innumerable	10/31/30	0
5	Brug.	1/27/30	Innumerable	11/4/30	0
6	Big.	4/21/30	Innumerable	10/22/30	0
7	Bel.	4/8/30	200	10/16/30	0
8	Bla.	12/18/29	Innumerable	2/6/30	9
9	Bur.	5/5/30	Innumerable	9/30/30	8
10	Bar.	3/18/30	Innumerable	6/11/30	0
11	Bell.	3/11/30	Innumerable	5/28/30	68
12	Bonn.	10/23/29	Innumerable	5/8/30	14
13	Bec.	11/21/29	Innumerable	3/11/30	19
14	Cla.	6/2/30	Innumerable	10/3/30	4
15	Cut.	5/7/30	Innumerable	9/19/30	6

COMMENTS: In this group of chronic Arthritics the outstanding clinical feature, as stated by the patients, was the marked relief from symptoms of auto-intoxication. They were more active and alert, both bodily and mentally, were capable of doing more work efficiently. Jointwise, however, we could not draw any definite conclusions, as we were not solely depending on one particular treatment but a combination of all accepted methods to control the disease.

The most promising field for the therapeutic usefulness of Lipid-soaps and a more direct means of evaluating its proper field of activity would be the use of it in active infections of the intestines such as enterocolitis, summer diarrhoea, etc. Our experience in this respect being very limited (3 cases of enteritis and 5 cases of chronic enterocolitis) we reservedly make the statement that Na Lecithids have a useful field of activity in such ailments of the intestinal tract. We reproduce here three photomicrographs of the stool smears of one of our enteric cases.

CONCLUSIONS.

1. Lecithin soap manifests specific bactericidal properties upon intestinal *Streptococcus* and *B. Coli*.

2. Lecithin soap is useful as a detoxifying agent and intestinal bactericide in arthritic cases.

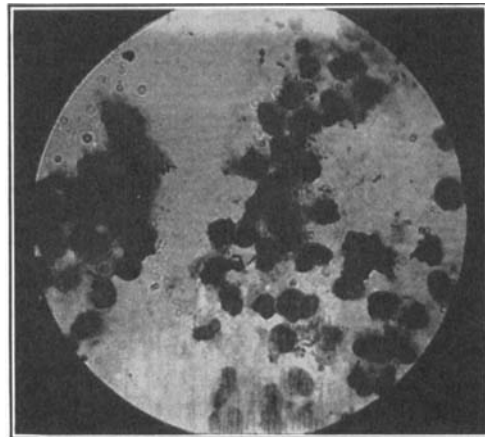


Fig. 3.—Shows the condition two weeks later with reappearance of some of the normal stool flora and an occasional diplococcus or chain. The persistence of pus cells is indicative of the fact that the disease is by no means yet cured.

REFERENCES.

- (1) Eggerth, Arnold H., *J. Gen. Physiol.*, 10, 147-160 (1926).
- (2) Eggerth, Arnold H., *J. Exp. Med.*, 46, 671-687 (1927).
- (3) Eggerth, Arnold H., *Ibid.*, 49, 53-62 (1929).
- (4) Eggerth, Arnold H., *Ibid.*, 50, 299-313 (1929).
- (5) Eggerth, Arnold H., *Ibid.*, 53, 27-36 (1931).
- (6) Spencer, R. R., "Public Health Reports," 45, 1354-1360 (1930).
- (7) Larson, W. P., and Nelson, E. N., *Proc. Soc. Exptl. Biol. Med.*, 21, 278 (1927).