Attempts to recultivate the typical organism from monkeys experimentally infected with the cultures and developing poliomyelitis, were successful with Berkefeld N filtrates and suspensions prepared from the nervous tissues of such animals.

The cycle of the disease has been demonstrated in monkeys inoculated with cultures. The organism has been developed from an invisible to a visible state and the typical disease induced in the experimental animal, in the brain and spinal cord of which the organism again returns to the invisible filterable stage.

It appears that the poliomyelitis virus has multiplied *in vitro*. The organisms cause pathological effects that can be augmented by successive passage of the culture material through a series of monkeys. Notable in this respect is the shortening of the incubation period.

The question of a possible life cycle and other biological considerations of the microbic agent are in the course of study. Additional inoculation experiments and investigations pertaining to morphology and virulence of cultures will be reported in the future.

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A Rapid Method for Determining Lactic Acid in Stools.

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In cultures, bacteria break down sugar into fatty acids, alcohol, lactic acid, methane, hydrogen and carbon dioxide. We have determined the amounts of the fatty acids occurring in human stools with various diets and under the conditions of constipation or catharsis.[1] Since lactic acid is produced in cultures in large amounts, this work was undertaken to answer the question whether lactic acid is present in stools in amounts comparable to those found for volatile fatty acids.

The isolation of lactic acid from stools could be carried out by extracting with ether or if a suitable precipitant were found which would free the filtrate from interfering substances, the filtrate might be directly oxidized. By means of the mercuric sulphate precipitation of West,[2] one can obtain from stool water clear filtrates prac-

[1] Grove, E. W., Olmsted, W. H., and Koenig, Karl, *J. Biol. Chem.*, 1929, **85**, 127.

[2] West, E. S., Scharles, F. H., and Peterson, V. L., *J. Biol. Chem.*, 1929, **82**, 137.

tically free from nitrogen and almost free from interfering substances.[3] The filtrate from the mercury precipitation must be further treated by the usual copper sulphate and lime precipitation. Applying the method of Friedemann, Cotonio and Shaffer,[3] we found that the amounts of oxidizable material in such filtrates is small as shown by the fact that 100 cc. of filtrate, representing from 5 to 10 gm. of stool, require in the lactic acid apparatus less than 50 cc. of 0.01 N potassium permanganate to show a permanent excess of permanganate. As a matter of fact, there was obtained only 0.2 to 0.5 mg. of sulphite binding substance from the above mentioned amount of material.

To obtain filtrates that do not give a biuret test, we found it necessary to use 2 volumes of a 30% mercuric sulphate solution in 10% sulphuric acid to one volume of stool. The dilution of the precipitated stool was found to be important. Thus to one volume of stool was added 2 of mercuric sulphate solution. The mercury was precipitated with 10% alkali, usually 2 to 3 volumes being necessary and the final dilution with water was to 10 volumes. Table I shows that with this dilution the best return of added zinc lactate was obtained. Furthermore, this dilution made possible rapid filtration through ordinary filter paper.

The use of relatively large amounts of stool and the resulting larger amounts of mercuric sulphate solution determined our choice of the alkali used to precipitate the mercury salt. As suggested by West,[2] barium carbonate is the ideal alkali to precipitate mercury but the huge amount of this salt required to precipitate the volume of mercuric sulphate solution necessary in this work prohibited its use. For the same reason and especially because of the evolution

TABLE I.

Showing the Dilution of Precipitated Stool Influences the Recovery of Added Lactic Acid. 2 volumes of 30% mercuric sulphate solution to 1 volume of stool. Total volume 100 cc.

Amount of stool precipitated	Sulphite binding substance expressed as lactic acid	Added zinc lactate expressed as lactic acid	Total lactic acid found*	Recovery
Gm.	Mg.	Mg.	Mg.	%
5	.36	1.21	1.27	75
7	.45	1.21	1.50	87
10	.53	1.21	1.53	82
14	.68	1.21	1.52	71
20	.82	1.21	1.62	66

*Sulphite binding substances expressed as lactic acid.

[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

of gas, sodium bicarbonate was not used. A 10% solution of sodium hydrate was found to be most satisfactory.

Of importance is the reaction of the stool during and at the end of precipitation. While the alkali is being added, the stool must be very vigorously stirred to prevent islands of strong alkali. For the same reason, the solution of sodium hydrate must be added slowly. The best recovery of added lactate was obtained when the reaction at the finish of the precipitation by alkali remained strongly acid to congo red paper. We suspect that the recovery of added lactate would have been better if it had been possible to use a weaker alkali.

Our method is as follows: To 100 gm. of stool, 200 cc. of a 30% solution of mercuric sulphate in 10% sulphuric acid was added. After passing through an ordinary kitchen sieve, 200 gm. portions of stool mixture were weighed out. From a burette, a 10% solution of sodium hydroxide was slowly added while stirring vigorously. At the end of the precipitation the reaction of stool was always just acid to congo red paper. The mercuric sulphate was added to stools immediately after defecation in order to sterilize them. Precipitation was done within a few hours, for if stools are allowed to stand in contact with the strongly acid mercuric sulphate solution a long time, the amount of sulphite binding substances is increased. The precipitated stool was made up to a liter and filtered. The filtrate was made more strongly acid by adding 1 cc. of 10% sulphuric acid. What mercury remained was precipitated by hydrogen sulphide. The excess gas was blown off with air saturated with moisture. After filtration, enough powdered copper sulphate was added to make a 2.5% solution[4] and calcium hydroxide was added until the solution was alkaline. After the alkaline copper precipitation had been allowed to stand in a refrigerator over night, the filtrate from the precipitation was oxidized by the method of Friedemann, Cotonio and Shaffer.[3]

100 cc. of filtrate was used for each determination. Determinations were made in duplicate and at the same time duplicate oxidations of pure solutions of zinc lactate were always run. The filtrate was neutralized by using phenol red as an indicator, after which 5 cc. of 2 M phosphoric acid and 10 cc. of 10% manganese sulphate were added. Oxidation was carried out with 0.01% potassium permanganate solution. We found, in agreement with Friedemann and Kendall,[5] that the optimum concentration of phosphoric acid is between 0.06 and 0.10 M.

[4] Ronzoni, Ethel, and Wallen-Lawrence, Zonja, *J. Biol. Chem.*, 1930, **74**, 363.

[5] Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

TABLE II.

Date and Subject	Weight of Stool	Wt. of Fraction of Stool	Lactic Acid* in Fraction	Added Lactic Acid	Total Lactic Acid* Found	Recovery	Total Lactic Acid* in Stool
	gm.	gm.	mg.	mg.	mg.	%	mg.
2/11/30-P	78	3.12	.389	1.22	1.47	88	9.7
2/13/30-P	252	7.46	.432	1.23	1.57	92.3	14.5
2/17/30-P	171	5.76	.528	1.23	1.57	85	15.7
2/25/30-P	200	6.40	.731	1.24	1.72	80	22.8
2/20/30-P	234	7.14	.391	1.21	1.36	80	12.9
2/28/30-P	142	5.04	.179	1.21	1.28	91	5.04
3/ 4/30-P	105	3.98	.095	1.21	1.21	93	2.5
3/ 5/30-P	289	8.12	.383	1.21	1.36	81	13.6
3/13/30-P	116	6.25	.276	.605	.828	91	5.1
3/14/30-O	107	7.14	.528	1.21	1.51	81	22.5
3/17/30-P	110	5.00	.140	1.21	1.16	84	3.08
3/18/30-O	184	5.00	.320	1.21	1.38	88	11.8*
3/18/30-P	106	3.33	.185	1.21	1.16	81	5.95

*Sulphite binding substances expressed as lactic acid.

In our own stools while taking a mixed diet, we could find but a few milligrams of sulphite binding materials (Table II).

The recovery of zinc lactate added to these stools amounted to 80 or 90%. This is in agreement with the results of Cori and Buchwald,[6] who used the same method in recovering lactic acid from bodies of frogs. Recovery of lactic acid from solutions of pure zinc lactate were $96 \pm 2\%$. In answer to the question, do small amounts of sulphite binding material arise from the oxidation of traces of lactic acid in stools, the following experimental facts should be considered. If one allows a stool to stand in contact with the acid mercuric sulphate solution for a period of several days, the amounts of sulphite binding substance will be doubled. Or if the stool filtrate is made alkaline and volatile substances are distilled off by steam, the sulphite binding substances are increased as much as 10 to 15%. However, if the stool filtrate is made acid and volatile acids distilled off, the sulphite binding substances are of the same value as before distillation. The data in Table I show that aliquot portions of stools when precipitated and oxidized do not give rise to aliquot amounts of sulphite binding materials. One can hardly conclude that we are dealing with sulphite binding substances arising from the oxidation of lactic acid alone.

Cori[7] has shown that lactic acid is absorbed at a much slower rate from the intestinal canal of the rat than similar amounts of the

[6] Cori, Carl F., and Buchwald, K. W., *J. Biol. Chem.*, 1931, **92**, 367.

[7] Cori, G. T., *J. Biol. Chem.*, 1930, **87**, 13.

TABLE III.

	Sterilization	Wt. of Fraction of Stool	Lactic* Acid in Fraction	Added Lactic Acid	Total Lactic Acid* Found	Recovery
	min.	gm.	mg.	mg.	mg.	%
A. 50 gm. stool plus 129 mg. lactic acid	0	5	.17	12.9	.18	0.08
B. 50 gm. stool plus 129 mg. lactic acid	30	5	.17	12.9	10.73	82.00

*Sulphite binding substances expressed as lactic acid.

various sugars. It is with difficulty, however, that our results can be explained on the basis of complete absorption, for lactic acid has been found in the stools of infants.[8, 9] The obvious explanation is that the intestinal flora of the adult destroy lactic acid. This conclusion is confirmed by the incubation for 24 hours at 37°C. (Table III) of lactic acid added to stool before and after sterilization. The lactic acid added to a stool incubated for 24 hours disappears. The usual recovery was found when the lactic acid was incubated with a sterilized stool. Kendall, Friedemann and Ishikawa[10] have shown that bacteria in the resting state destroys lactic acid. This is particularly true of the colon bacillus group.

Conclusions. 1. A rapid method for determining lactic acid in stools is presented. 2. Only a few milligrams of sulphite binding material was found in the study of the stools of 2 normal adults. 3. Evidence is presented that lactic acid is destroyed by intestinal bacteria.

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Complement Fixation in Variola and Vaccinia.

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Certain writers have drawn attention to the application of the

[8] Gerstley, J. R., Wang, C. C., and Wood, A. A., *Am. J. Dis. Child.*, 1930, **39**, 487.

[9] Gerstley, J. R., Wang, C. C., and Wood, A. A., *Am. J. Dis. Child.*, 1930, **39**, 729.

[10] Kendall, A. I., Friedemann, T. E., and Ishikawa, M., *J. Infect. Dis.*, 1930, **47**, 186.