gonococcus which were fatal for mice in large doses. Because of the instability of these materials, the preparations were lyophilized by means of a Flosdorf-Mudd apparatus.[6] This procedure not only stabilized, but also concentrated the preparations, which were complex mixtures of metabolic products and autolysed cells. A regenerated lyophilized ascitic fluid broth culture of the gonococcus, containing no viable organisms, was employed in our studies. It is this type of preparation to which we give the name "toxin."

Although ascitic fluid broth cultures of several of our strains of the gonococcus killed mice, the results reported herewith concern only one particular strain, the "Le D." This strain was isolated during March, 1937, from the knee-joint of a patient with gonococcal arthritis.

After determining that the intraperitoneal injection of 0.2 cc of the regenerated lyophilized "toxin" killed mice (20 to 30 gm) consistently, a study was made of the relative toxicities and therapeutic values of various preparations of sulfanilamide. A 2 per cent. solution of the compound in 0.85 per cent. NaCl solution was found to be most satisfactory for our purposes. The sulfanilamide was administered intraperitoneally in two doses, one of 10 mg immediately after the injection of the "toxin" and a second of 20 mg 5 hours later.

After inoculation, the control mice usually died within 18 hours; a few lived longer than 24 hours. The coat became rough; the mice were listless and remained in a crouched position until death. Generally, diarrhoeae developed shortly after inoculation and the temperature became subnormal. The eyelids were often stuck together with a mucopurulent exudate.

The mice treated with sulfanilamide became cyanotic, ataxic or spastic and markedly opisthotonic. The reactions from the drug subsided in less than an hour, after which time the mice presented, to a lesser degree, some of the symptoms observed in the controls. When the drug became effective, the treated mice improved gradually, the majority recovering within 48 hours.

TABLE 1
THE THERAPEUTIC EFFECT OF SULFANILAMIDE IN MICE INOCULATED WITH GONOCOCCAL "TOXIN"

Group	No. of mice injected	Treated No.	Treated Died	Untreated No.	Untreated Died
1 ..	15	10	0	5	5
2 ..	15	10	0	5	5
3 ..	13	9	0	4	4
4 ..	16	10	0	6	6
5 ..	8	5	0	0	3
6 ..	60	50	4	10	10
Total .	127	94	4(4.3 per cent.)	33	33(100 per cent.)

Summary		Per cent. protected	Standard error
	Mice with sulfanilamide .	95.7	±2.1
	Mice without sulfanilamide	0	

Results obtained with intraperitoneal doses of 10 and 20 mg of sulfanilamide administered immediately and 5 hours, respectively, after the intraperitoneal injection of 0.2 cc of lyophilized and regenerated gonococcal "toxin" ("Le D" strain).

[6] E. W. Flosdorf and S. Mudd, *Jour. Immunology*, 29: 389, 1935.

The results obtained by averaging 6 tests are given in Table 1. Six groups of mice were used in this particular study, the number of mice per group varying from 8 to 60. The total number was 127, of which 33 were controls. All the controls died within 24 hours. Only 4, or 4.3 per cent., of 94 treated mice failed to be protected. Of these, 2 lived for 4 days after treatment.

In summary, gonococcal "toxin" (a term used to describe the regenerated lyophilized ascitic fluid broth culture of the gonococcus, containing no viable cells, used in the present studies), which killed mice following its intraperitoneal injection, was produced from several strains of *Neisseria gonorrhoeae*. In the present report the results with one strain only, the "Le D," are described.

Mice injected with lethal amounts of the gonococcal "toxin" were protected from death by the administration of adequate therapeutic doses of sulfanilamide.

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THE FEASIBILITY OF PRESERVING NEOPLASTIC CELLS IN THE FROZEN STATE[1]

STUDIES on the resistance of single-celled or small multicellular animals and plants to extreme cold are reviewed by Heilbrunn,[2] who points out that survival after freezing may be due to factors which inhibit ice crystal formation. Ice crystals injure the cell both mechanically and by withdrawing water from the protoplasm. It is generally believed that slow freezing is associated with the formation of large crystals and is more injurious than rapid freezing.

Experiments on the resistance of the more labile neoplastic cells of mammals to freezing have yielded different results in the hands of different investigators. Carcinoma cells have been kept alive by Ehrlich[3] for two years at −8° C. This temperature does not completely solidify the tissue. Ehrlich has also found that the growth of carcinoma cells is retained after exposure to −30° C. for 48 hours. Michaelis[4] has successfully transplanted Jensen's tumor after it had been exposed to liquid air for a half hour. His obser-

[1] These investigations have been supported by the International Cancer Research Foundation, the Lady Tata Memorial Trust and a Fund for the Study of Leukemia.

[2] L. V. Heilbrunn, "An Outline of General Physiology," Philadelphia and London, W. B. Saunders Company, 1937.

[3] P. Ehrlich, *Zeitschr. f. Krebsf.*, 5: 65, 1907.

[4] L. Michaelis, *Med. Klin.*, 5: 204, 1905.

vations have been confirmed by Moore and Walker[5] and Gaylord.[6] Little consideration has been given to the rate of freezing or thawing, and the tissues have not been kept in the frozen state for long periods.

We have observed recently[7] that slow freezing is less injurious to neoplastic cells than is rapid freezing, and cells that were destroyed by rapid freezing survived slow freezing. At the time these observations were made, the authors were not aware of the similar results obtained by Rahn[8] with rotifers, nematodes and tardigrades. Rahn has found that these animals, in the active state, survived slow cooling to −253° C. but were killed when frozen rapidly.

The rate of deterioration of neoplastic cells in the frozen state is very slow, and the results shown in Table I indicate that they can be stored for long periods at −70° C.

TABLE I

RESULTS OF INOCULATIONS OF FROZEN AND THAWED MOUSE NEOPLASMS

Cell	Length of time kept at −70° C.	Route of injection	No. of mice		Average incubation period	Average length of life after inoculation
			inoculated	+		
	(days)				(days)	(days)
Lymphoid leukemia (Strain Akf 5)	8	s.c.	6	6	12	28
	86	s.c.	2	2	13	16
	147	s.c.	4	4	13	17
	440	i.v.,s.c.	4	4	15	25
Myelocytic leukemia (Strain Ar 117)	20	s.c.	7	5	19	42
	99	s.c.	6	5	17	58
	281	i.v.,s.c.	6	6	9	56
	440	s.c.	2	2	18	
Chloroleukemia (Strain S1b 351)	6	i.v.	4	1	24	42
	447	i.v.,s.c.	5	0		
	13	i.v.	4	1	21	36
	94	i.v.	5	0		
	1	i.v.,s.c.	5	4	14	25
Monocytic leukemia (Strain S2)	2	s.c.	4	4	14	23
	52	s.c.	4	3	13	20
	337	i.v.,s.c.	3	2	10	20
	430	i.v.,s.c.	4	3	15	25
Sarcoma (Strain S.3172)	1	s.c.	4	4	13	56
	151	s.c.	4	4	13	61
	448	s.c.	4	4	13	70
Carcinoma (Strain Afb 601)	2	s.c.	4	4	14	63
	98	s.c.	4	4	17	75

Abbreviations used in the table: s.c. = subcutaneously; i.v. = intravenously.

Technic: The tissue to be frozen was removed immediately after the animals were killed and was placed in a Petri dish on a chilled ice plate. It was minced with scissors in the presence of a small amount of Tyrode's solution. The minced tissue was distributed in small test-tubes (9 × 75 mm), approximately 0.5 cc in each, and the tubes were sealed with a blast lamp. The sealed tubes were immersed in alcohol at 0° C. and small pieces of solid C_2 were dropped in the alcohol at such a rate that the temperature dropped from 0° C. to −70° C. during approximately one hour. The frozen tissue was stored under alcohol in a 12-gallon thermos bottle containing large pieces of solid CO_2. The tissues to be injected were thawed rapidly by shaking the tube in water at 37° C. This was found to be less injurious to the tissue than slow thawing.

Epithelium of chicken trachea was preserved in the frozen state in a similar manner. Many cilia were actively motile after 2, 16 and 327 days.

All strains of neoplasms tested could be preserved without difficulty except for the myeloid cells of a transmissible chloroleukemia (Strain S1b 351), but these, too, were found to resist freezing when large fragments of spleen, with no Tyrode's solution, were frozen.

The possibility that transmission by frozen cells is due to a virus present in the cells is very unlikely.[7] The results of an experiment suggested by W. A. Barnes are noteworthy in this connection. Cells irradiated while in the frozen state at −70° C. with 4000 r of x-ray were completely inactivated. This dose of x-ray does not injure viruses at room temperature, and it is not likely to injure them at −70° C.

Summary: All neoplastic cells so far tested could be preserved by freezing, and the virulence of those tested after a year at −70° C. had not altered.

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[5] J. E. S. Moore and C. E. Walker, *Lancet*, 174: 226, 1908.

[6] H. R. Gaylord, *Jour. Infect. Dis.*, 5: 443, 1906.

[7] C. Breedis, W. Barnes, and J. Furth, *Proc. Soc. Exp. Biol. and Med.*, 36: 220, 1937.

[8] Rahn, quoted by Heilbrunn.[2]

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