TABLE II.

Dilution	Days alive									Survivors reinoculated (43rd day)	Days alive after reinoculation				Survivors
	2	3	5	7	8	10	13	14	15		2	3	4	14	
1-20	4	2								0					—
10-2	2	2		1					1	0					—
10-3			2	2	1					1		1			0
10-4				2	1	2	1			0					—
10-5								1	1	4		2	1	1	0
Saline										6	4	1	1		0

the disease occurred uniformly in all inoculated animals. The survivors were inoculated subsequently with known virus and succumbed to the disease. None proved to be immune.

Mice were inoculated with graduated dilutions of virus. Survivors of the sublethal doses were available for testing. These animals were reinoculated with lung emulsion of the lowest dilution 43 days after the original inoculations. The results are recorded in Table II.

Uninoculated cagemates of sick and moribund mice did not contract the disease. These animals, nevertheless, demonstrated no immunity to virus inoculation.

Large quantities of virus were injected subcutaneously and intraperitoneally into rabbits weekly for 8 weeks. Antiserum was mixed with virus for 30 minutes and inoculated intranasally one week after the last injection. The activity of the virus was not neutralized. Neither was previous intraperitoneal injection of antiserum effective in protecting mice against the virus.

This report describes the unusual immunological phenomenon of an invariably fatal clinical infection caused by a virus that lies dormant in the same host until stimulated by serial passage of lung emulsion or of physiological saline inoculated intranasally. No evidence of immunity to the latent virus has been demonstrated.

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Effect of Iodoacetate and Malonate on Respiration of Sea Urchin Sperm.

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Since Lundsgaard[1] discovered that iodoacetate in small concentrations inhibited the fermentation of glucose by yeast without

affecting its respiration, numerous papers have been published on the inhibitions produced by iodoacetate (of oxidation enzymes and of proteolytic enzymes), the inhibiting effect being probably due to combination with active sulfhydryl groups of the protein component of the enzymes. In some instances, concentrations of iodoacetate sufficient to inhibit oxidation reactions have had no effect on oxidations produced by bacteria[2] but in no case has there been reported increased respiration due to iodoacetate. The inhibition of succinate oxidation by malonate was discovered by Quastel and Whetham;[3] malonate was used as a specific inhibitor for this oxidation, though a number of investigators have reported that it inhibits also other oxidations.[4, 5, 6] Malonate seems to be utilized by moulds[7] and some bacteria;[8] in no case has it been reported that malonate is oxidized or increases the respiration of animal tissues. On studying the metabolism of sea urchin spermatozoa, we have found that both iodoacetate and malonate, used at the concentrations required for their inhibiting effect, increase their respiration.

Effect of Iodoacetate. 0.001 *M* iodoacetate dissolved in sea water, neutralized, and brought to the pH value of sea water, increased the oxygen consumption of sea urchin sperm soon after its addition (Fig. 1). This increase was due neither to oxidation of acetate (acetate produced no increase in the O_2 uptake of sea urchin sperm) nor to the action of liberated iodine (iodine inhibited the respiration). The increased O_2 uptake seems to involve the whole respiratory process, for it was accompanied by a similar increase in the CO_2 output. The R.Q. of sea urchin sperm, which was 1.08, remained approximately the same on addition of iodoacetate (1.12), the increase in the O_2 uptake in these experiments being 216% and the increase in CO_2 output 242% (Table I). Since the respiration of sea urchin sperm, like the sperm from other animals, increases on dilution, the effect of iodoacetate on sperm at three different dilutions was studied. Iodoacetate added to thick suspensions of sperm (Sperm I) increased the O_2 consumption to 277%; on approximately fourfold dilution (Sperm II), the increase was diminished to 215%; on further dilution of approximately eightfold (Sperm III) it was

1 Lundsgaard, E., *Biochem. Z.*, 1930, **217**, 162.
2 Barron, E. S. G., and Friedemann, T. E., *J. Biol. Chem.*, 1941, **137**, 593.
3 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1925, **19**, 525.
4 Greville, G. A., *Biochem. J.*, 1936, **30**, 877.
5 Weil-Malherbe, H., *Biochem. J.*, 1937, **31**, 299.
6 Baumann, C. A., and Stare, F. J., *J. Biol. Chem.*, 1940, **133**, 183.
7 Walker, T. K., Subramanian, V., and Challenger, F., *J. Chem. Soc.*, 1927, 3044.
8 Aubel, E., *Compt. Rend. Acad. Sci.*, 1921, **173**, 179.

only 93% (Table II). Iodoacetate at this concentration had no effect on the respiration of sea urchin fertilized eggs; at higher concentrations (0.03 *M*) Runnström[9] reported 34% inhibition, observed only after 30 minutes following addition of iodoacetate. As a rule, there is the same delay in the inhibiting effect in other cells.[10]

Effect of Malonate. Malonate (0.03 *M*) added to sea urchin sperm increased their respiration, like iodoacetate, soon after addition, the increase being not only in the O_2 uptake but also in the CO_2

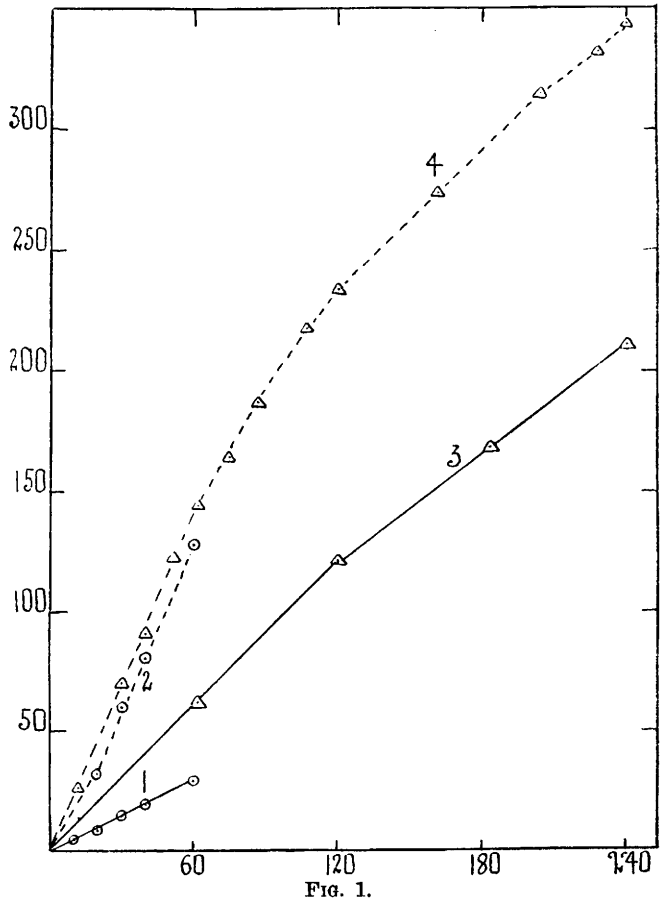

FIG. 1.

Effect of iodoacetate on the O_2 uptake of sea urchin sperm. Abscissa, time in minutes. Ordinate, O_2 uptake in mm^3.

1—Control (heavy suspension).
2—Heavy suspension with iodoacetate (0.001 *M*).
3—Dilute suspension.
4—Dilute suspension with iodoacetate.

9 Runnström, J., *Biol. Bull.*, 1935, **49**, 351.

10 Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem.*, 1933, **100**, 155.

TABLE I.
Effect of Iodoacetate (0.001 *M*) and of Malonate (0.03 *M*) on the Respiratory Quotient of Sea Urchin Sperm.

	O_2 uptake per hr, mm^3	CO_2 output per hr, mm^3	R.Q. $CO_2 : O_2$
Control	51.5	55.7	1.08
Iodoacetate	161.7	180.8	1.12
Control	40.0	45.0	1.12
Malonate	119.0	125.0	1.05

TABLE II.
Effect of Iodoacetate (0.001 *M*) on the Respiration of Sea Urchin Sperm and Eggs (fertilized).
Each number is the average of 5 experiments. Temp. 25°.

Cell	O_2 uptake per hr: Control, mm^3	O_2 uptake per hr: $CH_2ICOONa$, mm^3	Increase, %
Sperm I	24	90.5	277
" II	51.5	161.7	215
" III	121.0	234.0	93
Eggs I	33.0	30.0	None

output. The R.Q. thus remained unchanged (Table I). As in the iodoacetate experiments, the increase in the O_2 uptake diminished on dilution of the sperm. Thus, in thick suspensions of sperm (Sperm I, Table III) malonate increased the O_2 uptake by 200%; on about threefold dilution (Sperm II) the increase was 170%; on eightfold dilution the increase was only 69%. Succinate, which is oxidized by the sperm, increased the O_2 uptake by 35%; addition of malonate plus succinate gave not an additive increase but only the increase due to malonate. Whether this was due to inhibition of succinate oxidation by malonate was not determined. In sharp contrast to the effect on sperm respiration, malonate added to fertilized eggs inhibited slightly their O_2 consumption (8%) (Table III). Oxidation of malonate by sperm seems improbable not only because its complete oxidation would result in a change of the R.Q. of sperm (R.Q. for complete oxidation of malonate, 1.50) but also because, as already indicated, acetate, the decarboxylation product of malonate, does not increase the O_2 uptake of sperm.

Discussion. Three facts bear consideration in these experiments: (1) that iodoacetate and malonate increase equally the O_2 uptake and the CO_2 output of sea urchin sperm; (2) that the effect is immediate, contrary to the delayed effect when they act as inhibitors of cell oxidations; (3) that the percentage increase diminishes on dilution of sperm. These facts have led us to assume that the respiration

TABLE III.
Effect of Malonate (0.03 *M*) on Respiration of Sea Urchin Sperm and Eggs (fertilized).
Each number is the average of 5 experiments. Temp. 25°.

Cell	O_2 uptake per hr: Control, mm³	O_2 uptake per hr: Malonate, mm³	Increase, %
Sperm I	40	120	200
" II	88.4	238	170
" III	100.2	169	69
" succinate	135	171	26
Eggs I	44.5	40.7	None

of sea urchin sperm is normally inhibited by sulfhydryl groups present in the sperm fluid. The respiration would then be increased by a decrease of sulfhydryl concentration, accomplished partly by dilution, or by abolition of the activity of the sulfhydryl groups, which seems to be brought about by its combination with iodoacetate, and possibly with malonate. It is well known that thiol compounds react rapidly with halogen acids under physiological conditions.[11, 12] The presence of sulfhydryl groups in the sperm fluid was detected by the characteristic color reaction with nitroprusside. The respiration of sperm, high in lower animals, is greatly diminished in mammals. It is possible that the respiration of mammalian sperm is kept at low levels by the inhibiting action of sulfhydryl compounds; iodoacetate would then increase their respiration.

Conclusion. Iodoacetate and malonate added to sea urchin sperm in concentrations sufficient to produce inhibition of respiration in other animal tissues increased the respiration, the increase being equally effective on the O_2 uptake and the CO_2 output. No change in R.Q. was therefore observed. It is postulated that sea urchin sperm fluid possesses a substance—probably a sulfhydryl derivative—which normally keeps the respiration at a low level. Dilution of this substance or its destruction by addition of iodoacetate or malonate would produce an increase of the whole respiratory process.

[11] Hellstroem, N., *Z. physik. Chem. Abt. A*, 1931, **157**, 242.
[12] Dickens, F., *Biochem. J.*, 1933, **27**, 1141.