chronic oral administration of carbon tetrachloride. 2. A supplement of methionine, but not of cystine or of choline, showed a protective effect against damage produced by feeding sodium selenate (selenium 20 p.p.m.). 3. This action was demonstrated only in the presence of alpha-tocopherol. The relationship is discussed briefly.

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Experimental Histoplasmosis. Susceptibility of Male DBA Line 1 Mice By Various Routes of Injection. (18120)

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It has recently been shown that, by intracerebral injection, male dba line 1 mice, 4-5 weeks of age, are uniformly susceptible to *Histoplasma capsulatum* and are apparently more so, by this route of injection, than several other strains(1,2). In the present report it will be shown that this strain of mice is more susceptible to infection with *Histoplasma* by this route of injection than by any other route.

Procedure. The mice used in these experiments were primarily dba line 1. In addition, a few Bar Harbor C 57 Black mice, lines 6 and 10, also were employed. All mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and were 4-5 weeks of age, weighing 10-18 g at time of injection. Altogether, in the experiments in which routes of injection were compared, 137 male and 60 female dba line 1 mice, 35 male and 34 female Bar Harbor C 57 Black, line 10, and 23 male and 23 female Bar Harbor C 57 Black mice, line 6 were injected with *Histoplasma*. In addition to the above animals, a few mice in each group were injected with saline alone to serve as controls. However, since it was found that, in general, male mice of both strains were more susceptible than females, the present report will deal only with the results obtained with male animals. The strain of *Histoplasma*, No. 155, and the method of preparation of the inoculum were the same as that used in previous studies of this series(1). Three routes of injection were employed; for intracerebral injection, each mouse was injected with 0.02 ml of a given dilution of a saline suspension of the yeast phase of the fungus or 0.02 ml of saline alone; for intravenous injection, each animal was given 0.1 ml of the saline suspension of the fungus or 0.1 ml of saline alone; for intraperitoneal injection, each animal was given 0.5 ml of the saline suspension of the fungus or 0.5 ml of a suspension of the fungus in 5% mucin, or 0.5 ml of saline or mucin alone. The mucin suspension was prepared according to the method of Milzer and Levine(3). Equal parts of a 1-25 or a 1-50 saline suspension of the yeast phase of the fungus and the 5% mucin suspension were then mixed to give a 1-50 or a 1-100 dilution of the fungus in mucin. Serial dilutions were then prepared from this suspension with sterile physiological saline.

The estimation of the number of viable organisms actually injected, cultures of tissues obtained at autopsy, and other procedures followed were the same as those previously reported(1). A description of the gross lesions present at autopsy and the results of

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2. Howell, A., Jr., and Kipkie, G. F., *Am. J. Trop. Med.*, in press.

3. Milzer, A., and Levine, E. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 16.

TABLE I. Results, in Terms of Mortality, Obtained in a Series of Experiments in Which Male dba Line 1 Mice Were Injected by Specified Routes with a Saline or Mucin Suspension of the Yeast Phase of a Single Strain of *Histoplasma capsulatum* at Different Dose Levels.

		Route of injection							
		Intracerebral		Intravenous		Intraperitoneal			
						Organisms suspended in saline		Organisms suspended in 5% mucin	
Exp. No.	Dilution employed	Estimated dose per inj.*	No. of deaths†	Estimated dose per inj.*	No. of deaths†	Estimated dose per inj.*	No. of deaths†	Estimated dose per inj.*	No. of deaths†
I	1/100	319	3/3	3186	0/3	7965	3/3	13500	3/3
II	1/50	678	—	6774	1/5	16935	4/5	17500	5/5
	1/100	339	4/5	3387	1/5	8468	0/5	8750	2/5
	1/200	169	5/5	1694	1/5	4234	0/5	4375	1/4‡
	1/400	85	5/5	847	0/4	2117	0/5	2187	0/5
	1/800	42	5/5	423	—	1059	—	1094	—
	Saline	0	0/5	0	0/3	0	0/5	0§	0/5
IV	1/50	176	—	—	—	—	—	2400	2/6
	1/100	88	5/5	—	—	—	—	1200	—
	1/200	44	6/6	—	—	—	—	600	—
	1/400	22	6/6	—	—	—	—	300	—
	1/800	11	4/6	—	—	—	—	150	—
	1/1600	6	4/6	—	—	—	—	75	—
	1/3200	3	1/6	—	—	—	—	38	—
	1/6400	1	4/6	—	—	—	—	19	—
	Saline	0	0/4	—	—	—	—	0	—
Total No. of animals	Inj. with a suspension of *Histoplasma*		52/64		3/22		7/23		13/28
	Inj. with saline alone		0/9		0/3		0/5		0/5

* Expressed in thousands of organisms.
† No. of mice which died spontaneously before the 30th day after injection.
‡ One additional animal is not included in the tabulation as it died on the 4th day after injection of a generalized peritonitis, the result of traumatic perforation of the intestine.
§ Injected with 5% mucin alone.

the microscopic examination of sections from all tissues taken at autopsy will be presented later.

Results. The results, in terms of mortality obtained, of the inoculation of male dba line 1 mice by each of the three routes employed are presented in Table I.

A comparison of the death rates obtained immediately reveals sharp differences, depending on the route of inoculation. Pooling the results of all experiments for a single route of injection, it can be seen that an intracerebral injection, for example, of 1000 to 340,000 organisms, resulted in the spontaneous death, within 30 days after injection, of 52 of 64, or 81.3%, of the mice injected by this route. Intraperitoneal injection, however, of 2,000,000 to 17,000,000 organisms, suspended in saline, resulted in the death of only 7 of 23, or 30.4%, within the same interval. The addition of mucin for intraperitoneal injection, within the same dose range, increased the percentage of spontaneous deaths to 46.4% (13 of 28).

Not only were marked differences observed in the per cent of mortality obtained, depending on the route of injection, but also the time by which any given percentage of the animals were dead varied with the route (Fig. 1). By the fourteenth day following intracerebral injection, 47 of 64, or 73.4%, of the mice were dead; by intraperitoneal injection in saline only 2 of 23, or 8.7%; and, by intraperitoneal injection in mucin, only

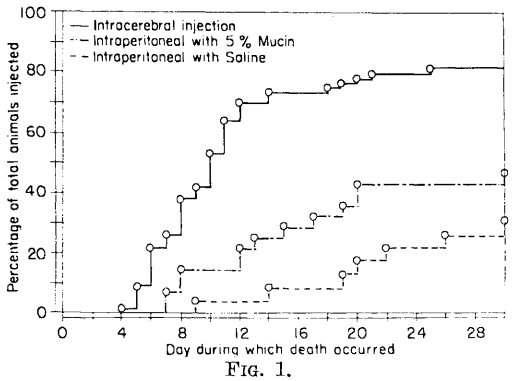

FIG. 1.

Cumulative death rates observed among male dba line 1 mice within 30 days of injection with varying doses of the yeast phase of *Histoplasma capsulatum*.

7 of 28, or 25.0%, were dead. Similar results were obtained with the Bar Harbor C 57 Black mice. For example, 9 of 11, or 81.8%, of line 6 died following intracerebral injection of 44,000 to 350,000 organisms, while only 5 of 12, or 41.7%, died following injection with 4,000,000 to 8,000,000 organisms suspended in mucin. The same doses resulted in the death of 9 of 14, or 64.3%, of line 10 mice following intracerebral injection, and 7 of 12, or 58.3%, following intraperitoneal injection in mucin. Although in the latter group almost as high a percentage were killed by intraperitoneal injection in mucin as by the intracerebral route, the dosage necessary to produce this fatality rate, by intraperitoneal injection, was twenty to almost one hundred times that given intracerebrally. No control animal in any experiment died before the sacrifice date.

Histoplasma was recovered in culture from the brain and/or spleen of each mouse injected with the fungus in these experiments except 8. Of these 8, cultures from 6 were overgrown with contaminants. In one additional animal, the tissues were so badly autolyzed at autopsy that no cultures were made. The remaining animal was a dba line 1 mouse injected intraperitoneally with mucin.

Comment. The finding that the addition of mucin for intraperitoneal injection of the yeast phase of *Histoplasma* into mice increases the susceptibility of these animals to this fungus is in agreement with the recent report of Campbell and Saslaw(4). However, with the strains of *Histoplasma* employed by these authors, and with the strain used in this study, enormous doses of the fungus were required to produce a relatively high degree of mortality. Campbell and Saslaw, for example, found that an intraperitoneal dose of approximately 3,500,000 organisms of their Strain No. G-8 suspended in mucin were necessary to produce 66.6% to 85% mortality by the thirtieth day after injection, using White Swiss mice (Bagg strain). This same strain of *Histoplasma*, designated by us as No. 167, in doses of 7,000 to 115,000 organisms, by intracerebral injection, killed 26 of 30, or 86.6%, of male dba line 1 mice within thirty days after injection(5).

It has also been shown that dba line 1 mice are more susceptible to *Histoplasma* than White Swiss mice and that the time of death, after intracerebral injection, can be regulated by the dosage employed(1,2). Therefore, it would seem, from the data presented above, and from a comparison of the results obtained by Campbell and Saslaw (4) with that obtained by us in other experiments, that mice are much more susceptible to intracerebral injection with *Histoplasma* than to intraperitoneal injection in saline or mucin.

Summary. In a series of experiments it has been shown that male dba line 1 mice are relatively resistant to intravenous injection of the yeast phase of *Histoplasma capsulatum*. Intraperitoneal injection of enormous numbers of the organisms suspended in saline produced occasional deaths. Intraperitoneal injection of approximately the same numbers of viable organisms suspended in 5% mucin increased the death rate. Intracerebral injection, however, was far superior to any of the other routes employed, both with respect to the numbers of organisms necessary to produce death 30 days after injection and the percentage of fatalities obtained within this period of time.

4. Campbell, C. C., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.

5. Howell, A., Jr., and Kipkie, G. F., *J. Lab. and Clin. Med.*, 1950, v36, 547.

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