METHYLMERCURY UPTAKE FROM WATER AND FOOD BY AQUATIC ORGANISMS FROM DIFFERENT TROPHIC LEVELS

by

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Methylmercury uptake from water and food by aquatic organisms from different trophic levels.

ABSTRACT

Uptake of methylmercury (as CH_3HgCl) from water was studied in a unicellular green alga, Chlamydomonas reinhardtii, a cladoceran, Daphnia pulex, and a fish, Salmo gairdneri. In D. pulex and S. gairdneri, the relative uptake of mercury from water and food was compared to assess the significance of these two modes of mercury accumulation. Toxic concentrations of methylmercury were established for each species and subsequent experiments conducted at levels well below these concentrations. No difference in accumulation was observed between living and dead C. reinhardtii, suggesting surface adsorption. Uptake was directly related to concentrations of mercury in the medium, at least at concentrations to 20 ng Hg/ml.

In D. pulex and S. gairdneri the rate of mercury uptake from the water was higher than from the food, but the percentage uptake of mercury was higher from the latter source. In both species, uptake at mercury levels < 1 ng Hg/ml showed a linear relationship with mercury concentrations in the media, but at the higher levels, the uptake ratios gradually decreased. This suggests the operation of some mechanism, possibly increased mucus production at the respiratory surfaces and the gut epithelium, limiting mercury accumulation. Although aquatic organisms are capable of removing methylmercury from the water, it is concluded that organisms of higher trophic levels in

natural aquatic systems accumulate most of their mercury from food since methylmercury dissolved in water is present in extremely low concentrations (<1 ng/l).

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To my wife, Joke, I owe a great deal for her continuous support, encouragement and understanding throughout this study.

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INTRODUCTION

High levels of mercury (> 1 μg/g) in fish were first observed in the 1950's in Minamata Bay, Japan, and have since been found in various organisms from different parts of the world (Johnels et al., 1969; Wobeser et al., 1970; Uthe and Bligh, 1971). Most of the mercury in these organisms was in the form of methylmercury (Westöö, 1966, 1969; Jernelöv, 1969; Johansson et al., 1970), although mercury is often present only as inorganic mercury in original sources. This difference can now be attributed to methylation of inorganic mercury by microbial and chemical processes in aquatic sediments (Wood et al., 1968; Jensen and Jernelöv, 1969; Landner, 1971; Wood, 1974). In addition, there is some evidence that bacteria (e.g. Pseudomonas sp.) in the intestine of fish and on the external mucus are able to methylate inorganic mercury (Tonomura and Kanzaki, 1969).

Numerous experiments have shown that organisms rapidly remove various forms of mercury from water (Rucker and Amend, 1969; Jernelöv, 1970; Gillespie and Scott, 1971; Gillespie, 1972) and from food (Hannerz, 1968; Bäckström, 1969). However, quantitative data on the relative importance of uptake from these two sources are limited (Jernelöv and Lann, 1971). Several authors have suggested that mercury concentrations in organisms are related to the trophic level occupied by such organisms (Stock and Cucuel, 1934; Fimreite et al., 1971; Gavis and Ferguson, 1972). However, others have not observed this relationship (Hannerz, 1968; Copeland and Ayers, 1972).

The aim of this study was to evaluate the significance of both

water and food as sources of methylmercury (as CH_3HgCl) for three organisms: a unicellular green alga, Chlamydomonas reinhardtii; a cladoceran, Daphnia pulex and the rainbow trout, Salmo gairdneri. I determined the significance of the primary producer (C. reinhardtii) as a source of methylmercury for D. pulex and evaluated the contribution of mercury in the food to that accumulated by S. gairdneri. These data were compared with direct uptake of methylmercury from water by these organisms. Since these investigations, of necessity, had to be conducted at sub-lethal mercury concentrations, it was necessary to first establish lethal levels for each organism and then to conduct the uptake experiments at concentrations not toxic over the period of experimentation.

MATERIALS AND METHODS

Mercury concentration in all samples was determined by acid digestion, followed by flameless atomic absorption spectrophotometry. The spectrophotometric method was that described by Uthe et al. (1970), except that I used a flow-through system. The procedure involved complete digestion of tissue samples ($\leqslant$1 g) or water (75 ml) with 10 ml of concentrated HNO_3: HCl (4 : 1 v/v) in 100-ml flasks at 60 C. Samples were subsequently cooled to 0 C for 1 hr and oxidized with 10 ml 7% aqueous $KMnO_4$ (w/v), one drop of 30% H_2O_2, and brought to 100 ml with distilled water. Samples were transferred to polyethylene bottles in which they could be stored for at least one week without apparent loss of mercury.

A 50-ml aliquot from each digested sample was transferred to a 125-ml reaction flask. Manganese oxides were removed and mercury reduced by addition of 5 ml hydroxylamine hydrochloric acid and 5 ml 20% (w/v) stannous chloride. The mixture was stirred magnetically for 1 min and purged with a constant flow (300 ml/min) of dried purified air via the absorption cell of a Perkin-Elmer atomic absorption spectrophotometer (Model 290) into a flask where the mercury vapour was finally trapped with a saturated solution of potassium triiodide. Absorption was measured at 253.7 nm and values corrected for the reagent blanks and per cent recovery (94.4%). Average coefficient of variation between replicate samples with concentrations ranging from 5 - 50 ng Hg/ml was 4.3%; detection limit was 0.1 ng Hg/ml. One-way analysis of variance was used for all statistical comparisons.

Cultures of Chlamydomonas reinhardtii (wild type, mating strain 89, 90) were grown in sterilized Beijerinck's medium at 16 ± 1 C. Cultures were maintained on a 16 : 8 hr light - dark cycle in either cotton-stoppered 2800-ml Fernbach or 250-ml Erlenmeyer flasks on a reciprocating shaker under daylight fluorescent lamps producing 2.5 x 10^3 ergs/cm^2/sec.

The effect of methylmercury (as CH_3HgCl) on the growth rate of C. reinhardtii was measured in replicate 125-ml cultures over a range of 0 - 40 ng Hg/ml. Growth rates were monitored either spectrophotometrically (685 nm) or from cell counts obtained with a Coulter Counter equipped with a 50 μ aperture tube. To calibrate the spectrophotometer, algae were periodically collected on a membrane filter (HA Millipore, 0.45 μ), dried at 60 C for 24 hr and weighed. A relationship between absorbance, dry weight and cell number was established.

The relative importance of surface adsorption and active uptake of methylmercury by C. reinhardtii was investigated by comparing the rates of uptake in living and killed cultures. At the beginning of exponential growth (1.0 x 10^5 cells/ml), 1-liter cultures were killed with ethanol (6 - 7% v/v) and methylmercury added to these and living cultures. Initial mercury concentration was 20 ng Hg/ml. Periodically, 100-ml samples were taken from each culture, the cells separated from the medium by centrifugation at 4000 rpm for 10 min. and prepared for mercury analysis.

The uptake of methylmercury as a function of algal concentration and mercury concentration in the medium was also evaluated. At each

of five different cell concentrations six cultures were established. Each culture was exposed in the dark to one of six mercury concentrations ranging from 0.5 - 20 ng Hg/ml. After complete removal of the mercury from the medium, (15 mins - 10 hr, depending on initial mercury concentration), cultures were killed with ethanol, centrifuged and C. reinhardtii analysed for mercury content.

Daphnia pulex (de Greer), obtained from Deer Lake, British Columbia, were maintained in 14 C dechlorinated water in 20-l "Planktonkreisels" (Greve, 1968) on a mixture of C. reinhardtii and yeast cells. To establish the effect of methylmercury in the food on survival and reproduction of D. pulex, six triplicate groups of 10 adult female D. pulex of similar size and comparable numbers of eggs in their brood pouches were fed C. reinhardtii, previously cultured in methylmercury concentrations ranging from 0.5 - 20 ng Hg/ml. This food was given in a concentration which sustained maximum survival and reproduction of D. pulex (see Appendix I).

The effect of methylmercury in the water on survival and reproduction was measured by exposing adult females to water containing methylmercury in concentrations ranging from 0.5 - 20 ng Hg/ml. These concentrations were below the 96-hr medium tolerance limit (TL_m) for D. pulex (see Appendix II). A proportional diluter similar to that described by Mount and Brungs (1967) provided water of the desired mercury concentrations to the containers in which mesh-covered beakers with D. pulex were submerged. The animals were fed C. reinhardtii, previously cultured in methylmercury concentrations ranging from 0.5 - 20 ng Hg/ml. Experiments lasted for 14 days and

extended over two generations.

Uptake of methylmercury from the water was measured by transferring approximately 10,000 D. pulex into each of a series of 5-1 containers, continuously supplied with methylmercury at concentrations below those which affected their survival and reproduction (0.25 - 5 ng Hg/ml). Periodically, samples were removed, briefly washed with distilled water, and prepared for mercury analysis.

Mercury uptake from food by D. pulex was determined by feeding them C. reinhardtii (2.0×10^5 cells/ml) previously grown in methylmercury concentrations ranging from 0.25 - 5 ng Hg/ml. Periodically, samples of D. pulex were transferred to dechlorinated water, allowed to clear their guts and then analysed for mercury content. Feeding rates were determined as described by Sheldon and Parsons (1966). Percentage of mercury assimilated was calculated from mercury concentrations in C. reinhardtii and in D. pulex and feeding rate of D. pulex . Uptake experiments lasted for 72 hr.

Juvenile rainbow trout (Salmo gairdneri) weighing 2 - 3 g, were obtained from the Fish and Wildlife Branch Hatchery at Abbotsford, British Columbia. They were kept in 13 ± 1 C dechlorinated water and fed commercial trout food pellets each day in an amount equal to about 4 - 5% of their wet weight. Uptake of methylmercury from water was assessed with five groups of 20 trout. Each group was exposed in a flow-through system to one of five different concentrations of methylmercury ranging from 0.25 - 5 ng Hg/ml. These concentrations were below the 96-hr TL_m for rainbow trout (Appendix II). Four fish from each group were removed after 1, 2, 3, 6 and 12 weeks of continu-

ous exposure. Two fish were blotted dry, weighed and homogenized in a tissue grinder. The remaining two were homogenized after a thorough rinse with dechlorinated water and a brushing with 5% HNO_3 solution to remove mucus. Replicate 1-g samples of each homogenate were used for mercury analysis.

Methylmercury uptake from food was measured over a 12-week period by providing trout with food containing five different concentrations of methylmercury. Groups of 10 trout were placed in containers continuously supplied with dechlorinated water. The original intent was to feed the fish mercury-containing D. pulex. The trout's consumption of D. pulex, however, was slow and moreover, D. pulex gradually loses mercury when transferred to dechlorinated water. Consequently, mercury uptake using D. pulex as a food source could not be measured with sufficient accuracy. Therefore, the fish were fed mercury-containing pelleted food. Pellets previously soaked in five different concentrations of methylmercury were provided daily at a ration of 4 - 5% of the fishes wet weight. Mercury concentrations in the pellets on a dry weight basis were within 10% of those in D. pulex exposed for 72 hr to methylmercury ranging from 0.25 - 5 ng Hg/ml. Samples of two fish from each group were taken after 1, 2, 3, 6 and 12 weeks of feeding. Mercury concentrations in these fish were determined 24 hr after the last feeding.

Percentage withdrawal of mercury from water was calculated from the mercury concentration in the trout, that in the water, and the volume of water passing through the gill chamber (ventilation volume; see Appendix III for sample calculation). Percentage assimilation of mercury from the food was determined from food intake, mercury concentration in the food, and mercury concentration in the trout.

RESULTS

Exposure to methylmercury increased the lag phase in Chlamydomonas reinhardtii cultures. This was particularly evident at mercury concentrations exceeding 10 ng Hg/ml (Fig. 1). Relative growth constants (K) and mean generation times (T) were calculated as follows:

$$K = \frac{\log_e N - \log_e N_o}{t - t_o} = \frac{2.3\ (\log_{10} N - \log_{10} N_o)}{t - t_o}$$

and $$T = \frac{\log e^2}{K} = \frac{0.693}{K}$$

where N = cell concentration at time t, N_o = cell concentration at time t_o with t and t_o = time in days taken from the linear portion of the growth curve.

Growth constants for the control culture and cultures exposed to 1, 5, and 10 ng Hg/ml of methylmercury were 0.38, 0.35, 0.32 and 0.27 respectively. Corresponding mean generation times were 44.3, 47.8, 51.1 and 61.4 hr. Growth of C. reinhardtii cultures exposed to 20 ng Hg/ml was severely retarded, whereas those cells exposed to 40 ng Hg/ml were killed (Fig. 1). The effect of methylmercury on algal growth was reduced at higher cell concentrations (Fig. 2).

Uptake of methylmercury by living and killed C. reinhardtii exposed to 20 ng Hg/ml showed no difference during the first 10 hr (Fig. 3). The

Fig. 1. Growth of Chlamydomonas reinhardtii in media containing different initial concentrations of methylmercury (ng Hg/ml). (●), control; (○), 1; (△), 5; (■), 10; (□), 20; (▲), 40. Cultures were maintained at 16 ± 1 C under white light of 2.5 x 10^3 ergs/cm^2/sec on a 16 : 8 hr light - dark regime.

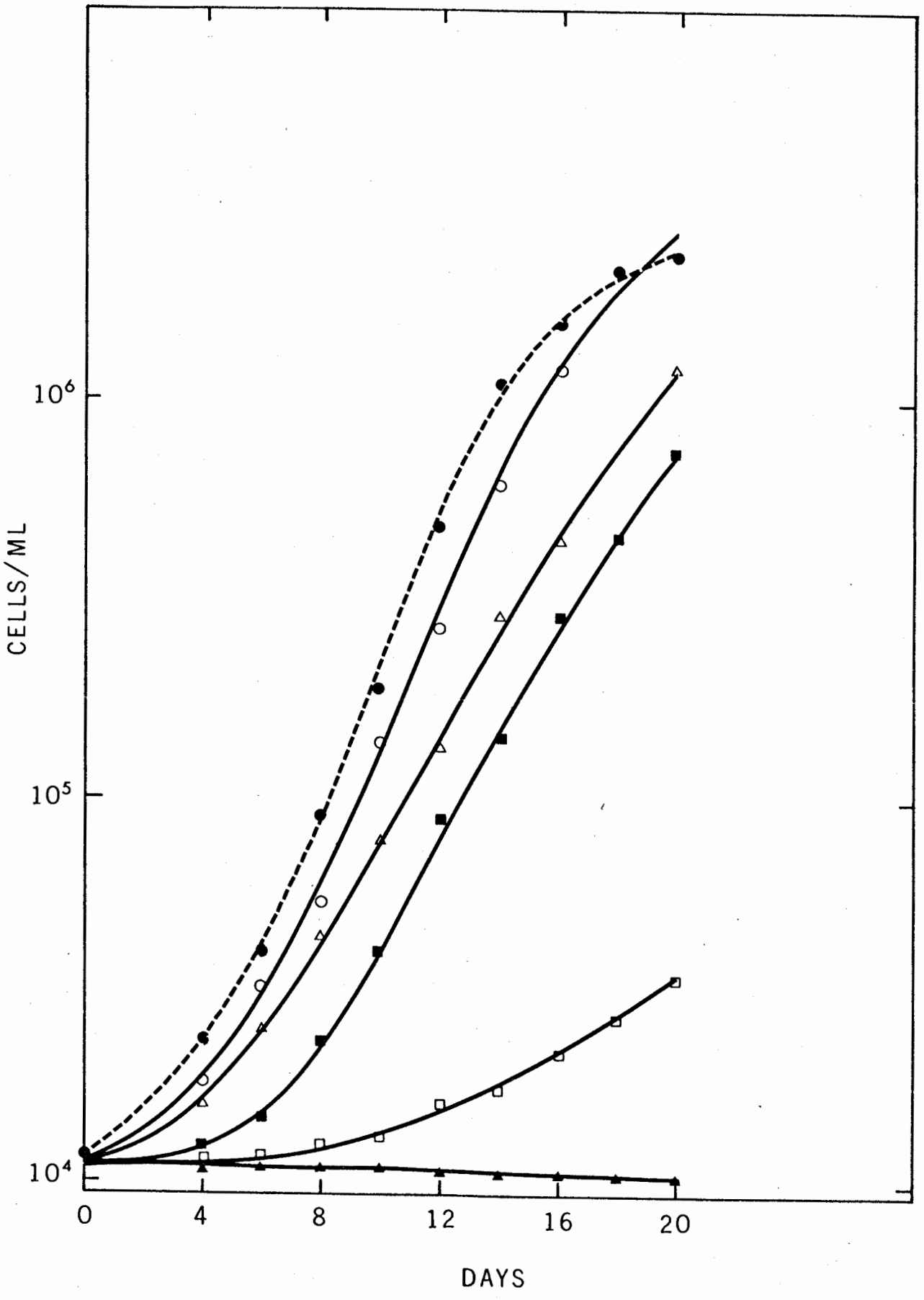

CELLS/ML
10^6
10^5
10^4
0
4
8
12
16
20
DAYS

Fig. 2. Growth of Chlamydomonas reinhardtii in relation to initial cell concentrations and methylmercury concentrations (ng Hg/ml). (●), control; (○), 1; (△), 5; (■), 10; (□), 20; (▲), 40. Cultures were maintained at 16 ± 1 C under white light of 2.5×10^3 ergs/cm^2/sec on a 16 : 8 hr light - dark regime.

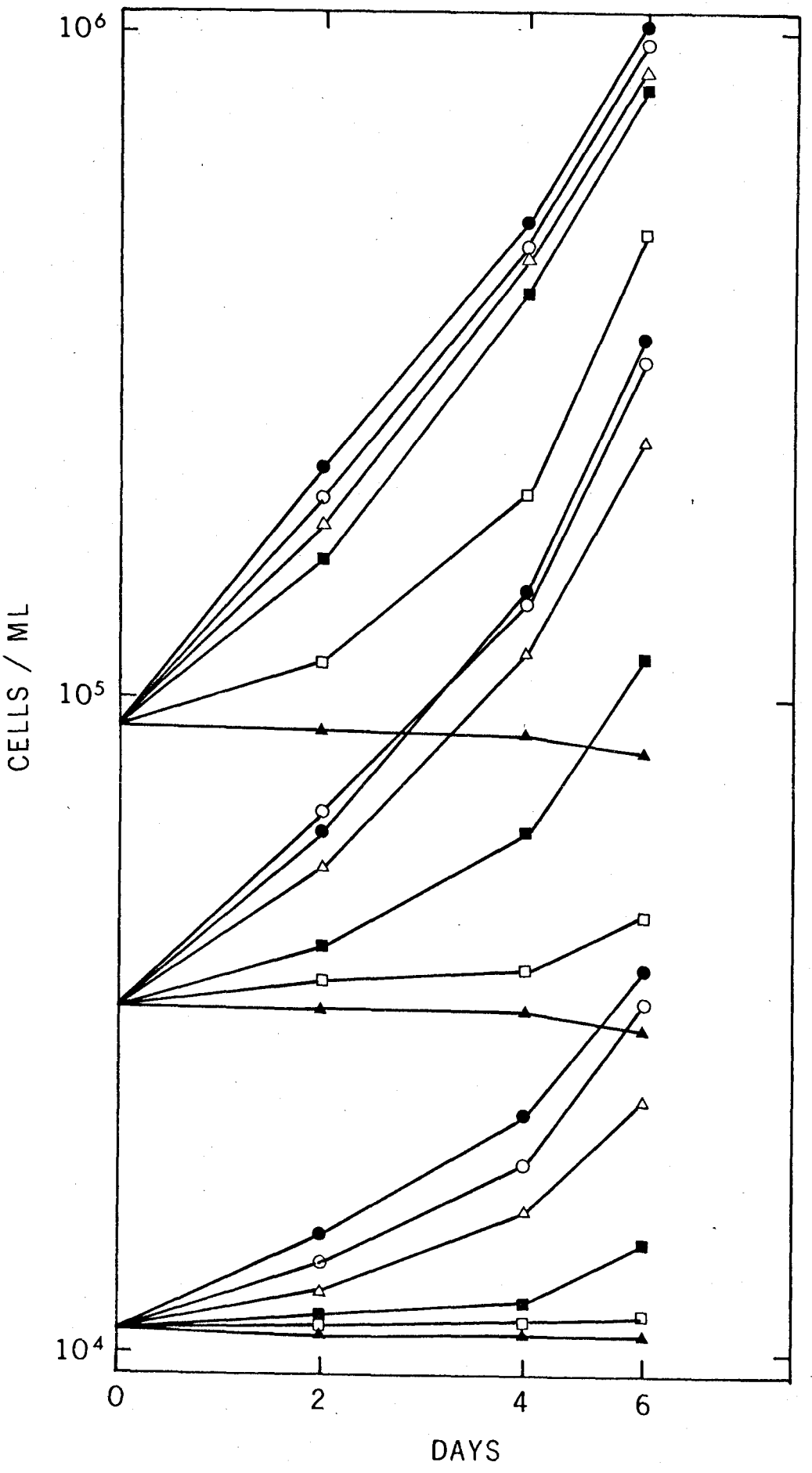
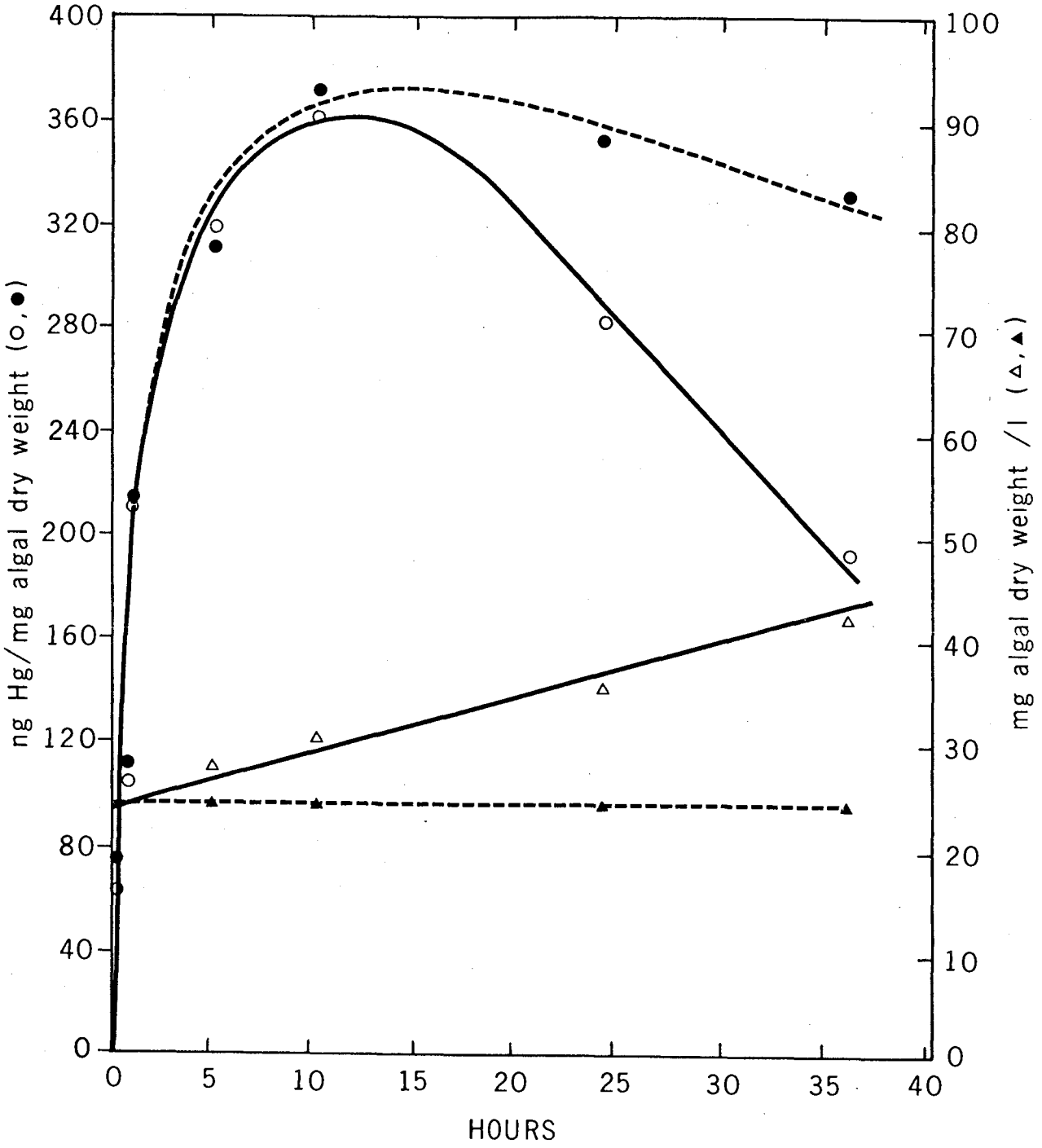

10^6
10^5
10^4
CELLS / ML
0
2
4
6
DAYS

Fig. 3. Uptake of methylmercury by living (o——o) and killed (●----●) cultures of Chlamydomonas reinhardtii. Cultures were exposed to an initial concentration of 20 ng Hg/ml. Living cultures were maintained at 16 ± 1 C under white light of 2.5 x 10^3 ergs/cm^2/sec on a 16 : 8 hr light - dark regime. Algal biomass (mg algal dry weight/l) in living (△) and killed (▲) cultures.


400
360
320
280
240
200
160
120
80
40
0
ng Hg/mg algal dry weight (o, ●)
100
90
80
70
60
50
40
30
20
10
0
mg algal dry weight /l (△, ▲)
0
5
10
15
20
25
30
35
40
HOURS

decreased mercury concentration in living cells after 10 hr is probably attributable to exhaustion of mercury in the medium. Uptake of methylmercury at different concentrations by C. reinhardtii increased linearly with the initial mercury concentration in the medium to 20 ng Hg/ml. Moreover, uptake per unit weight was inversely related to cell concentration (Fig. 4).

In experiments concerning methylmercury effect on survival and reproduction of D. pulex, when methylmercury was only available via the food, only C. reinhardtii previously exposed to 20 ng Hg/ml caused a significant decrease ($p < 0.05$) in survival of the F_1 generation and reproduction of both the F_1 and the parental generation (Table 1). When methylmercury was present in water as well as food, the effects were more pronounced (Table 2). In this case, each of the two highest concentrations significantly reduced survival of the parental generation ($p < 0.05$) and prevented its reproduction. Accordingly, in subsequent experiments on methylmercury uptake by D. pulex, concentrations < 10 ng Hg/ml were used.

Uptake of methylmercury by D. pulex from water was rapid and reached a maximum in 1 hr. Final concentrations in D. pulex were related to those in the water (Fig. 5). Similarly, mercury concentrations in D. pulex fed contaminated food were related to the algal mercury concentrations (Fig. 6). However, the percentage of mercury assimilated by D. pulex was inversely related to mercury concentration in the food (Table 3).

Uptake of methylmercury by trout was related to concentrations in the water (Fig. 7). Rate of uptake was high during the first three weeks, after which concentrations in the fish remained more or less con-

Fig. 4. Uptake of methylmercury by Chlamydomonas reinhardtii in relation to cell concentration (mg algal dry weight/l) and initial mercury concentration (ng Hg/ml). (□), 0.5; (●), 1.0; (▽), 2.5; (■), 5.0; (○), 10; (▲), 20. Cultures were exposed in the dark and killed after mercury in the medium was depleted.

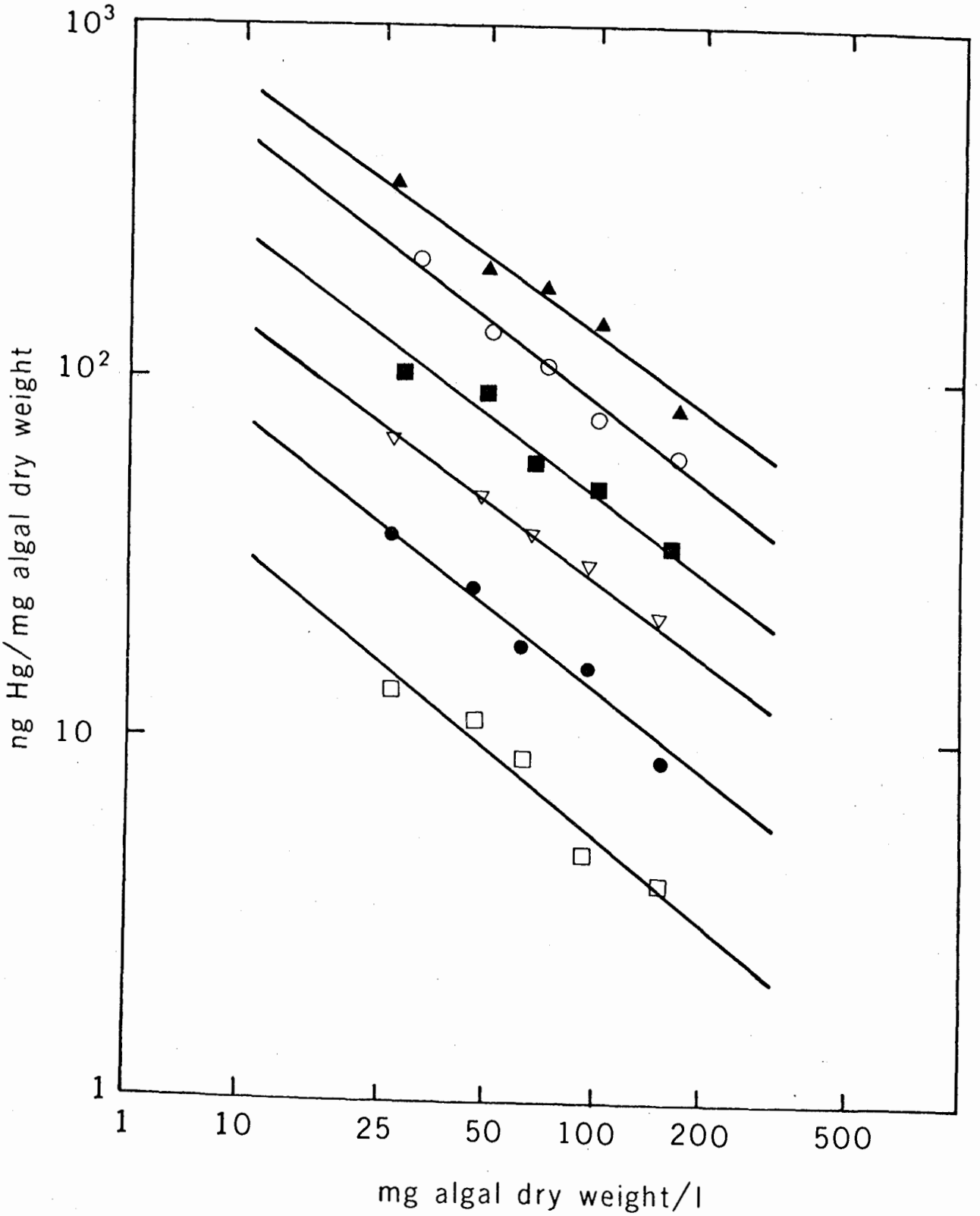

10^3
10^2
10
1
ng Hg/mg algal dry weight
1
10
25
50
100
200
500
mg algal dry weight/l

Table 1. Survival and reproduction of two generations of Daphnia pulex feeding on Chlamydomonas previously cultured in different concentrations of methylmercury. Values are the means of three samples.

Methylmercury conc. in Chlamydomonas (ng Hg/mg dry wt)	Calculated amount of methylmercury consumed[1] (ng Hg/mg dry wt of Daphnia/hr)	Parent generation		F_1 generation	
		Surv. %	Reprod. coeff.[2]	Surv. %	Reprod. coeff.
0	0.00	100	19	93	17
39	2.52	84	20	82	12
74	4.77	92	15	88	14
126	8.13	94	18	90	17
235	15.60	82	16	86	15
360	23.29	89	11	63	9

[1]) Based on a feeding rate of 64.48 µg algal dry wt/mg dry wt of Daphnia/hr.

[2]) Total number of juveniles produced divided by number of adults initially added.

Table 2. Survival and reproduction of two generations of Daphnia pulex feeding on Chlamydomonas previously cultured in different concentrations of methylmercury and simultaneously exposed to the same mercury concentrations in which the Chlamydomonas had been cultured. Values are the means of three samples.

Methylmercury conc. in water (ng Hg/ml)	Parent generation		F_1 generation	
	Survival %	Reprod. coeff.[1]	Survival %	Reprod. coeff.
0.0	95	16	100	19
1.0	97	17	94	16
2.5	85	15	71	12
5.0	88	19	82	17
10.0	67	0	-[2]	-
20.0	15	0	-	-

[1]) Total number of juveniles produced divided by number of adults initially added.

[2]) - No F_1 generation present.

Fig. 5. Uptake of methylmercury by Daphnia pulex from water in relation to mercury concentration (ng Hg/ml). (■), control; (□), 0.25; (▼), 0.5; (○), 1.0; (△), 2.5; (●), 5.0. Continuous-flow experiments conducted at 14 C. Values are means of three samples ± standard error.

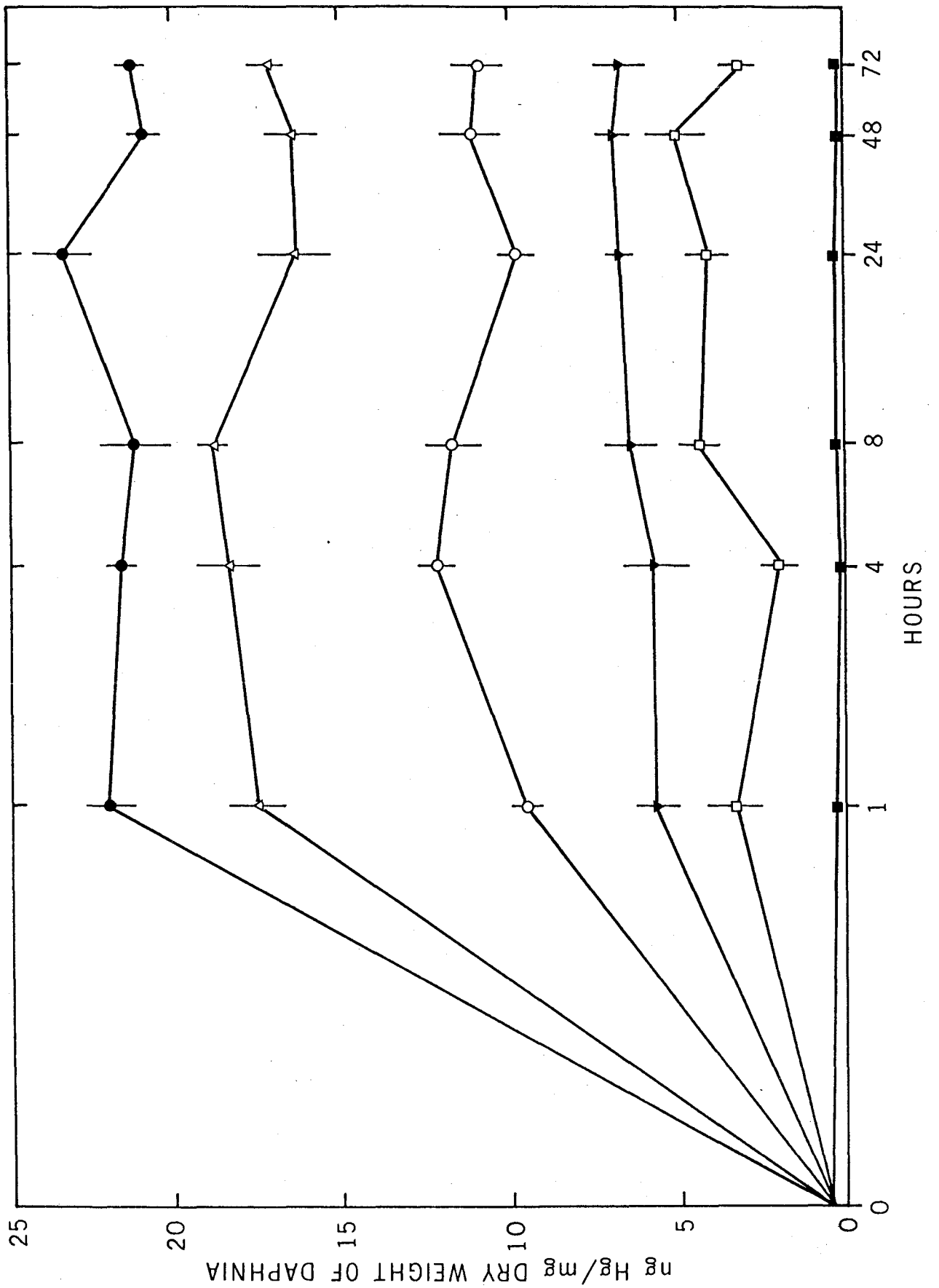

ng Hg/mg DRY WEIGHT OF DAPHNIA
25
20
15
10
5
0
0
1
4
8
24
48
72
HOURS

Fig. 6. Uptake of methylmercury obtained by Daphnia pulex from Chlamydomonas reinhardtii which had previously been exposed to different concentrations of methylmercury (ng Hg/ml). (■), control; (□), 0.25; (▼), 0.5; (○), 1.0; (△), 2.5; (●), 5.0. Values are means of three samples ± standard error.

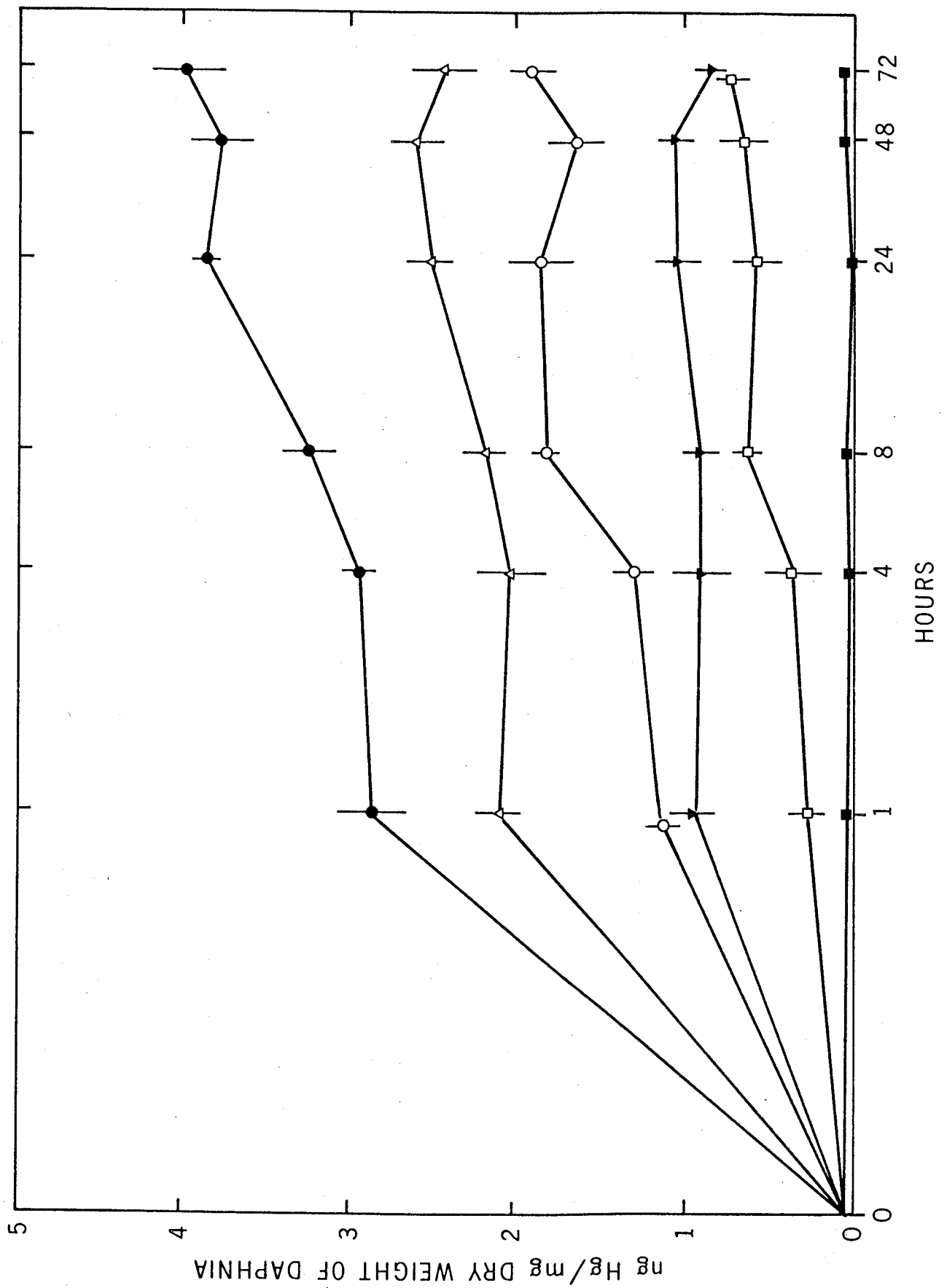
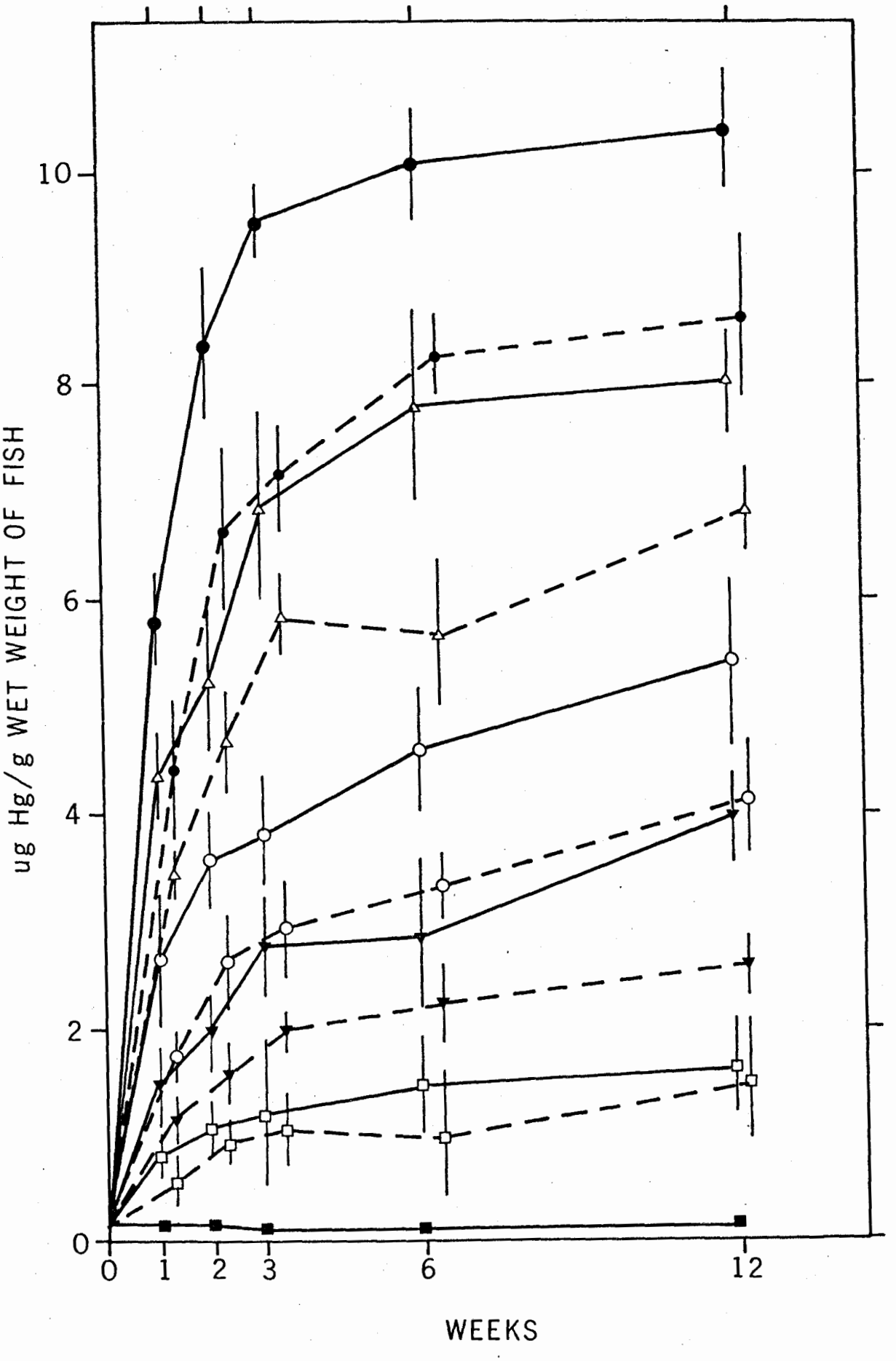

ng Hg/mg DRY WEIGHT OF DAPHNIA
0
1
2
3
4
5
HOURS
0
1
4
8
24
48
72

Table 3. Assimilation of mercury by Daphnia pulex feeding on Chlamydomonas containing different concentrations of methylmercury. Assimilation is expressed as a percentage of total mercury consumed during the feeding period[1]. Mean values of three samples ± standard error indicated.

Methylmercury concentration in algal cells (ng Hg/mg dry wt)	Duration of feeding (hours)					
	1	4	8	24	48	72
9	43.10 ± 2.58	19.39 ± 3.01	12.93 ± 1.07	4.33 ± 0.40	2.33 ± 0.35	1.79 ± 0.42
16	92.23 ± 1.45	21.84 ± 4.85	10.92 ± 1.21	4.17 ± 1.07	2.18 ± 0.10	1.07 ± 0.74
39	47.62 ± 3.96	13.89 ± 1.09	9.17 ± 0.64	2.97 ± 0.33	1.36 ± 0.17	1.04 ± 0.08
74	45.07 ± 3.14	10.74 ± 1.31	5.76 ± 0.40	2.23 ± 0.17	1.13 ± 0.09	0.71 ± 0.08
126	35.05 ± 2.46	9.07 ± 0.30	5.07 ± 0.31	1.95 ± 0.03	0.96 ± 0.05	0.68 ± 0.04

1) Based on a feeding rate of 64.48 μg algal dry wt/mg dry wt of Daphnia/hr

Fig. 7. Uptake of methylmercury by Salmo gairdneri from water containing different mercury concentrations (ng Hg/ml). (■), control; (□), 0.25; (▼), 0.5; (○), 1.0; (△), 2.5; (●), 5.0. Continuous-flow experiments conducted at 14 C. (——), mercury concentration in whole fish sample with mucus; (----), mercury concentration in whole fish sample without mucus. Mean of six samples ± standard error indicated.


ug Hg/g WET WEIGHT OF FISH
0
2
4
6
8
10
0
1
2
3
6
12
WEEKS

stant. Comparison of mercury concentrations in total fish with those in fish after removal of mucus indicated that mercury content of the mucus was higher than that of the tissues, except at the lowest concentration of mercury used (Fig. 7). Percentage withdrawal of mercury was inversely related to the mercury concentration in the water and also decreased with length of exposure (Table 4).

The amount of mercury assimilated during 12 weeks of feeding was positively correlated with the concentration of methylmercury in the food (Fig. 8). Percentage assimilation was inversely related to concentration in the food, and for each concentration decreased with time (Table 5). Comparison of Fig. 7 and 8 shows that accumulation of methylmercury from the water during the first three weeks, particularly at the higher concentrations, was more rapid than from the food. Only in the latter case, however, accumulation continued after this period. There was no difference in growth rate between controls and mercury-treated fish.

Table 4. Withdrawal of mercury by Salmo gairdneri from water containing different concentrations of methylmercury. Withdrawal is expressed as a percentage of total amount of mercury in water passing through the gill chamber during exposure time, based on a ventilation volume of 10 ml/min. Mean of six samples ± standard error indicated.

Methylmercury concentration in water (ng Hg/ml)	Exposure time (weeks)				
	1	2	3	6	12
0.25	8.46 ± 0.45	6.78 ± 0.75	6.38 ± 0.82	4.40 ± 1.57	5.49 ± 0.89
0.50	6.74 ± 0.92	6.63 ± 0.94	5.93 ± 0.46	5.61 ± 0.67	4.38 ± 0.43
1.0	5.46 ± 0.61	4.79 ± 0.66	4.32 ± 0.60	3.75 ± 0.28	3.58 ± 0.42
2.5	4.28 ± 0.18	4.19 ± 0.30	2.68 ± 0.14	2.58 ± 0.29	2.36 ± 0.08
5.0	2.69 ± 0.37	2.50 ± 0.28	2.20 ± 0.12	1.89 ± 0.22	1.47 ± 0.11

Fig. 8. Uptake of methylmercury by Salmo gairdneri from food containing different mercury concentrations (μg Hg/g dry wt). (■), control; (□), 3.4; (▼), 6.2; (○), 9.3; (△), 15.8; (●), 21.6. Food pellets were consumed at a daily ration of 4 - 5% of the fishes wet weight. Mean of six samples ± standard error indicated.

Table 4. Withdrawal of mercury by Salmo gairdneri from water containing different concentrations of methylmercury. Withdrawal is expressed as a percentage of total amount of mercury in water passing through the gill chamber during exposure time, based on a ventilation volume of 10 ml/min. Mean of six samples ± standard error indicated.

Methylmercury concentration in water (ng Hg/ml)	Exposure time (weeks)				
	1	2	3	6	12
0.25	8.46 ± 0.45	6.78 ± 0.75	6.38 ± 0.82	4.40 ± 1.57	5.49 ± 0.89
0.50	6.74 ± 0.92	6.63 ± 0.94	5.93 ± 0.46	5.61 ± 0.67	4.38 ± 0.43
1.0	5.46 ± 0.61	4.79 ± 0.66	4.32 ± 0.60	3.75 ± 0.28	3.58 ± 0.42
2.5	4.28 ± 0.18	4.19 ± 0.30	2.68 ± 0.14	2.58 ± 0.29	2.36 ± 0.08
5.0	2.69 ± 0.37	2.50 ± 0.28	2.20 ± 0.12	1.89 ± 0.22	1.47 ± 0.11

Fig. 8. Uptake of methylmercury by Salmo gairdneri from food containing different mercury concentrations (μg Hg/g dry wt). (■), control; (□), 3.4; (▼), 6.2; (○), 9.3; (△), 15.8; (●), 21.6. Food pellets were consumed at a daily ration of 4 - 5% of the fishes wet weight. Mean of six samples ± standard error indicated.

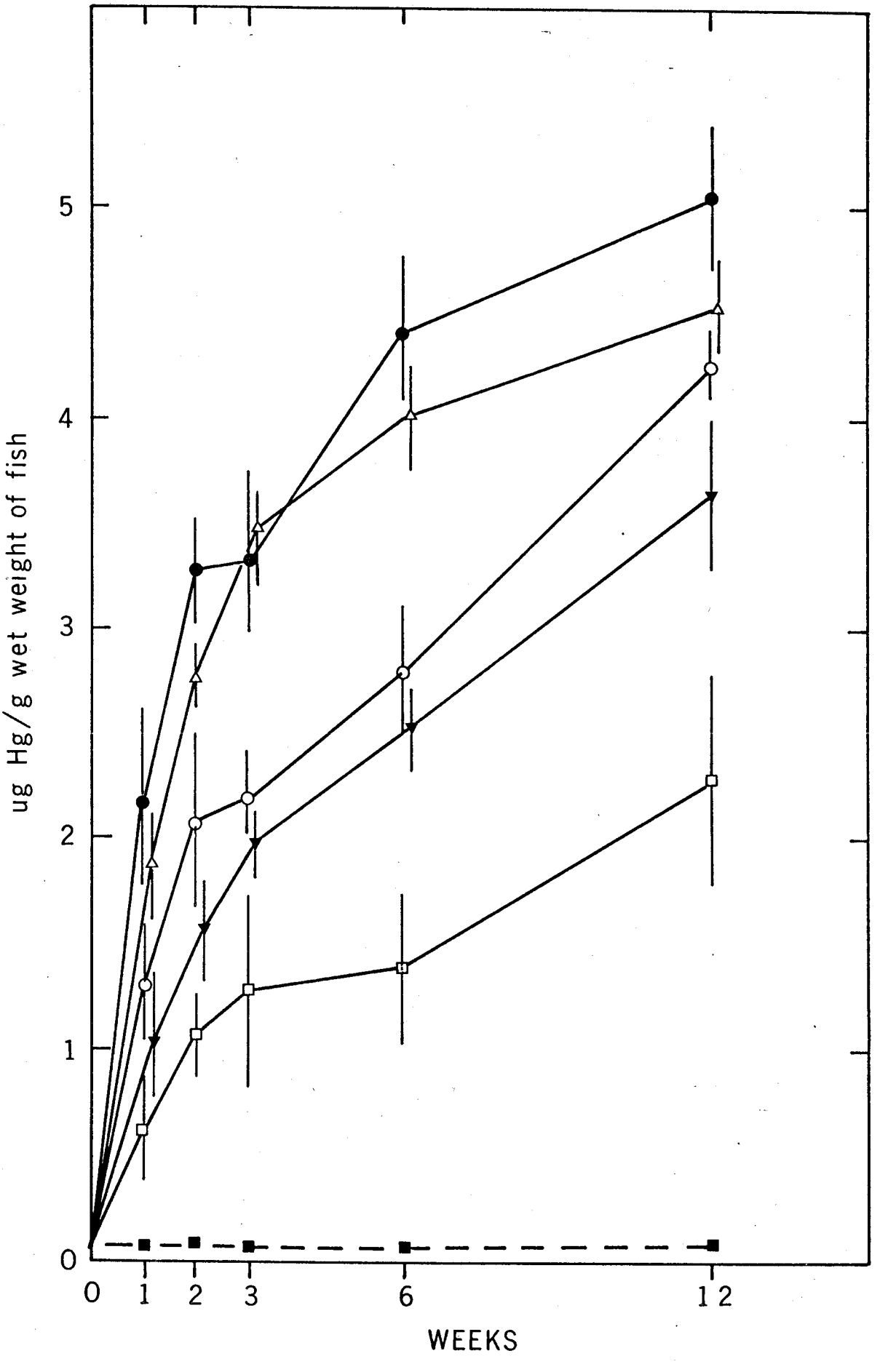

ug Hg/g wet weight of fish
0
1
2
3
4
5
0
1
2
3
6
12
WEEKS

Table 5. Assimilation of mercury by Salmo gairdneri from food containing different concentrations of methylmercury. Assimilation is expressed as a percentage of total amount of mercury ingested during the feeding period, based on a daily consumption of 4 - 5% of the fishes wet weight. Mean of six samples ± standard error indicated.

Methylmercury concentration in the food (μg Hg/g dry wt)	Duration of feeding (weeks)				
	1	2	3	6	12
3.4	71.11 ± 13.15	60.77 ± 8.33	52.48 ± 9.10	37.75 ± 4.34	31.44 ± 5.92
6.2	63.41 ± 9.97	46.36 ± 6.07	51.86 ± 2.97	36.22 ± 2.37	26.40 ± 2.30
9.3	52.44 ± 10.16	43.03 ± 8.08	36.20 ± 3.30	26.28 ± 2.79	20.73 ± 0.57
15.8	45.32 ± 5.27	32.89 ± 1.42	34.63 ± 2.01	22.67 ± 1.39	12.88 ± 0.63
21.6	38.02 ± 6.65	29.30 ± 1.91	24.48 ± 2.28	17.89 ± 1.20	10.56 ± 0.62

DISCUSSION

The observed delay in the onset of exponential growth of Chlamydomonas reinhardtii exposed to methylmercury in sublethal concentrations (Fig. 1), seems to be a characteristic effect of this metal on algae. It confirms the observation of Ben-Bassat (1972) for C. reinhardtii and Kamp-Nielsen (1971) for Chlorella pyrenoidosa, as well as those of Harriss et al. (1970) for a natural phytoplankton community. Both Kamp-Nielsen (1971) and Harriss et al. (1970) reported that the growth-inhibiting effect of methylmercury decreased with increasing cell concentration. My investigations yielded the same result (Fig. 2). These last observations suggest that the amount of mercury per cell is an important factor governing the effect of mercury on algal growth.

There were no differences in rate of methylmercury uptake between living and dead C. reinhardtii (Fig. 3), suggesting an accumulation by passive adsorption. The importance of surface adsorption in the uptake of mercury by algae and other aquatic plants has been emphasized by others (Hannerz, 1968; Glooschenko, 1969). As C. reinhardtii grown in Beijerinck's medium does not develop a mucoprotein sheath (J. Stein, personal communication), the first step in the accumulation probably is binding to the cell membrane. This would be facilitated by the high solubility of methylmercury in lipids and its low solubility in water ($P_{coeff.}$>100; Hughes, 1957). Whether methylmercury subsequently enters the cells and how it affects them is not known. In Chlorella pyrenoidosa, according to Kamp-Nielsen (1971), the primary effect of mercury bound to the membrane is a leakage of potassium, followed by phosphate. This would cause a reduction in metabolic activity and lead to reduced

autospore formation. Kamp-Nielsen (1971) also suggested that ions penetrate the cells and then exert additional effects by binding to the active sites of enzymes.

The relation of mercury accumulation in D. pulex to the concentration of methylmercury in water and food (Fig. 5 and 6), suggests an uptake mechanism involving an equilibrium reaction. If accumulation was solely determined by an equilibrium between mercury in water or food and the available binding sites in D. pulex, one would expect the uptake ratio (K_u), i.e. the ratio of maximum mercury concentration in D. pulex to the concentration of mercury in the source, to be the same for each of the five concentrations at which uptake was measured. This expectation rests on the assumption that the mercury accumulated by D. pulex was well below saturation levels. However, as can be seen in Table 6, the uptake ratios were similar only at lower mercury concentrations and gradually decreased with higher concentrations. Therefore, I conclude that exposure of D. pulex to a threshold concentration of methylmercury activates a mechanism that reduces further mercury uptake. In my experiments, the threshold concentrations were between 1 and 2.5 ng Hg/ml in water and between 16 and 39 ng Hg/mg in food. The reduced uptake occurred within 1 hr after exposure began.

The time required for maximum accumulation of mercury in trout (Fig. 7 and 8) was longer than in D. pulex, particularly when mercury was provided in food, but the patterns of uptake were similar. Uptake ratios in trout were similar only at the lower concentrations (Table 6), but they decreased at concentrations of methylmercury > 1 ng Hg/ml in the water or > 9.3 ng Hg/mg in the food. The mechanism responsible for

Table 6. Uptake ratios (K_u)[1] relating mercury accumulated by Daphnia pulex and Salmo gairdneri to methylmercury concentrations in water or food.

D. pulex				S. gairdneri				
Hg conc. in water (ng/ml)	K_u	Hg conc. in food (ng/mg)	K_u	Hg conc. in water (ng/ml)	K_u (fish with mucus)	K_u (fish without mucus	Hg conc. in food (ng/mg)	K_u
0.25	12.4×10^3	9	0.060	0.25	5.8×10^3	4.4×10^3	3.4	0.45
0.50	12.5 x ,,	16	0.061	0.50	6.1 x ,,	4.4 x ,,	6.2	0.43
1.00	11.3 x ,,	39	0.042	1.00	5.3 x ,,	3.6 x ,,	9.3	0.32
2.50	7.1 x ,,	74	0.030	2.50	2.9 x ,,	2.4 x ,,	15.8	0.24
5.00	4.3 x ,,	126	0.023	5.00	2.1 x ,,	1.6 x ,,	21.6	0.19

[1]) For Daphnia, the average for all 6 samples (taken at 1, 4, 8, 24, 48 and 72 hr) was used.

For Salmo exposed to Hg in the water the average value of samples taken at 3, 6 and 12 weeks was used.

For Salmo exposed to Hg containing food, only the 12 weeks sample was used.

this decrease in uptake ratio limited mercury accumulation to levels below those that would have been attained if uptake were solely a function of methylmercury concentration in water or food and numbers of available binding sites.

The question arises what may have caused this limitation in mercury uptake. In fish, mercury uptake from water occurs almost exclusively via the gills (Olson et al., 1973). Methylmercury at concentrations > 1 ng Hg/ml apparently inhibited this mode of uptake, but the mechanism involved is not clear. Presumably, uptake of methylmercury via the food parallels food absorption from the gut. An adverse effect of mercury on this last function is a theoretical possibility. As, however, in my experiments mercury treatment did not affect growth of the fish, it is highly unlikely that the relatively reduced mercury uptake from water or food at higher methylmercury concentrations is to be attributed to a decreased volume of water flowing through the gill chamber or a decrease in food utilization.

I hypothesize that the mechanism causing reduction of mercury uptake involves the production of mucus. Mucus-secreting cells are numerous in gill and gut epithelium. Mucus has a well-known affinity for methylmercury (McLeod and Pessah, 1973). Moreover, mucus excretion is stimulated by both inorganic and organic mercury (McKone et al., 1971; Rucker and Amend, 1969; Amend et al., 1969). In my experiments with trout, methylmercury in concentrations of 25 - 50 ng Hg/ml induced a visible increase in production of skin mucus. To my knowledge, data concerning the binding of methylmercury by intestinal mucus, have not been published. However, Clarkson (1971, 1973) reported that introduc-

tion in the food of an "artificial mucus" (a resin rich in SH-groups) was capable of reducing by more than 60% the intestinal absorption of methylmercury in mice. As mucus in the gut as well as in the gills is continuously produced and eliminated, it could provide a means to prevent a portion of the methylmercury from entering body tissues.

It is not clear whether the suggested role of mucus could also apply to D. pulex. The mechanism of methylmercury accumulation from water by D. pulex probably involves uptake across the respiratory surfaces, but the presence of mucus at these surfaces is uncertain. As D. pulex is a suspension feeder, its food uptake is accompanied by ingestion of considerable amounts of mucus produced by glands at the base of the fourth thoracic limbs and by the labral glands (Jørgensen, 1966). Thus, it seems likely that some methylmercury removed by D. pulex from food and water is bound to mucus and subsequently eliminated.

Two aspects of my investigation may be of particular relevance to the phenomenon of methylmercury accumulation by animals in a natural aquatic environment: uptake is apparently limited by mercury concentration in the medium and uptake from food is many times more efficient than from water (Table 4 and 5). Few data on total methylmercury concentrations in water exist and even less conclusive information is available on the partitioning of methylmercury between dissolved and particle-bound fractions (Jenne, 1973). My study and that of Burrows and Krenkel (1973) showed a rapid uptake of any dissolved methylmercury by organisms. Except for heavy industrial contamination of natural waters and sediments by methylmercury, such as occurred in Minamata Bay, Japan, concentrations of this compound in water are normally extremely low. For

example, Chau and Saitoh (1974) reported that lake water contained dissolved methylmercury in concentrations < 1 ng Hg/l. Furthermore, there is evidence that a variety of bacteria are able to demethylate methylmercury to the inorganic form (Spangler et al., 1973). As most of the methylmercury is present in bottom sediments, it is very likely that benthic organisms accumulate this compound to a considerable degree. This is supported by the observation that of all fish collected from major rivers and lakes throughout the U.S., bottom feeders such as catfish had the highest concentration of methylmercury (Henderson and Shanks, 1973). The occurrence of high concentrations of methylmercury in predatory fish from apparently uncontaminated waters (Johnels et al., 1967; Wobeser et al., 1970) is best explained by assuming that the mercury in these predators is acquired by consumption of benthic organisms or animals feeding on the benthos. In view of the relatively high concentration of methylmercury in these food sources and my observation that mercury uptake from the food is many times more "efficient" than from the water, I would conclude that the food chain is the major source of methylmercury accumulation in organisms of higher trophic levels.

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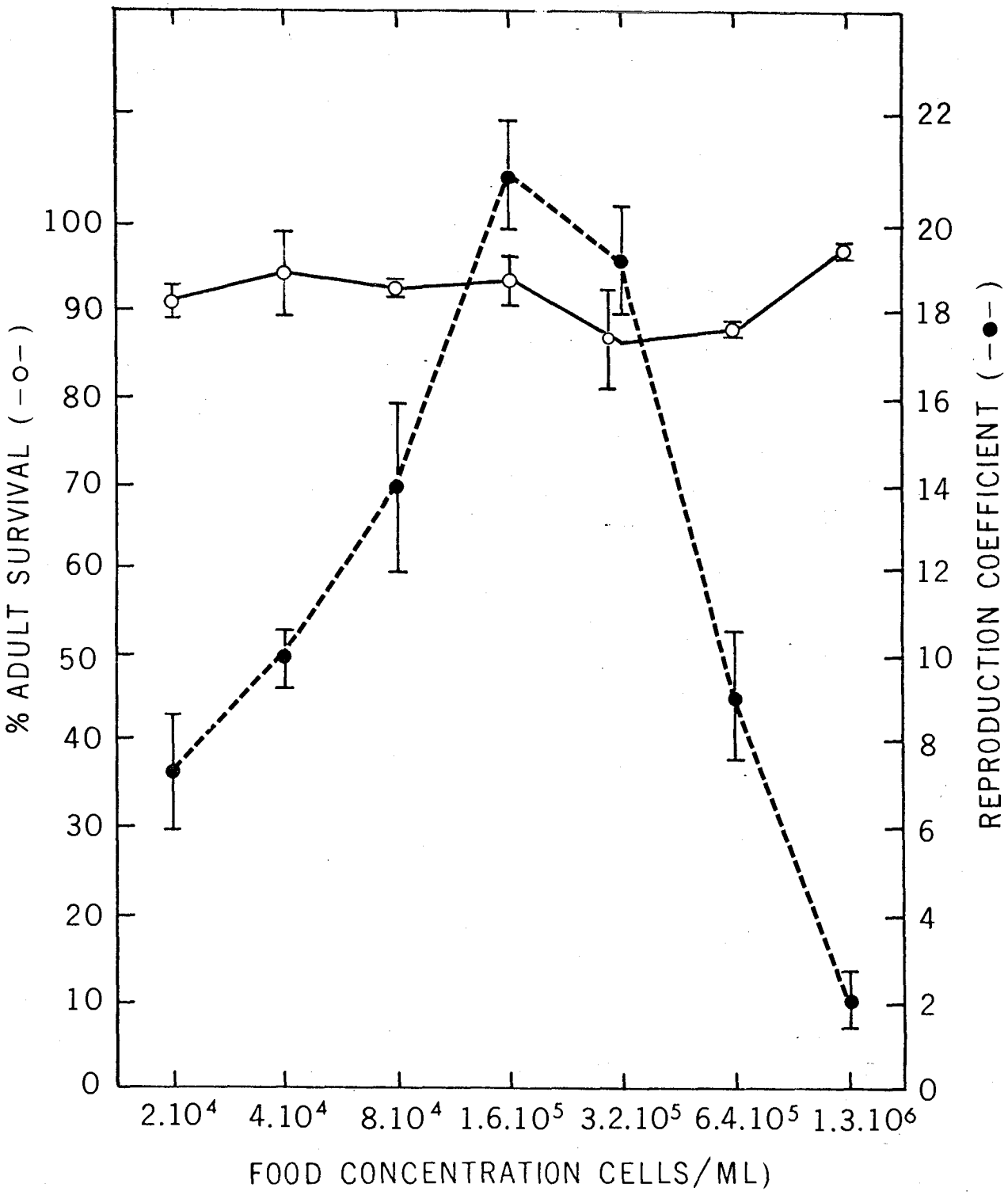
Appendix I. Determination of effect of food concentration on reproduction and survival of Daphnia pulex.

To establish the food concentration of Chlamydomonas reinhardtii yielding maximum survival and reproduction of D. pulex, adult female D. pulex were maintained in 250-ml darkened BOD bottles at one of seven different concentrations of C. reinhardtii. Water and food were renewed daily. After one week, all D. pulex were removed and separed according to size. Adult survival and reproduction coefficients were determined. The results indicate that food concentrations of approximately 2.0×10^5 cells/ml will give a maximum survival and reproduction (Fig. I).

Fig. I. The effect of food concentration (Chlamydomonas reinhardtii) on survival and reproduction of Daphnia pulex at 14 C. Mean values ± standard error indicated.


% ADULT SURVIVAL (-o-)
REPRODUCTION COEFFICIENT (-●-)
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20
22
2.10⁴
4.10⁴
8.10⁴
1.6.10⁵
3.2.10⁵
6.4.10⁵
1.3.10⁶
FOOD CONCENTRATION CELLS/ML)

Appendix II. Determination of the median tolerance limit (TL_m) for methylmercury (as CH_3HgCl).

Concentrations of methylmercury toxic to Daphnia pulex and Salmo gairdneri were determined to establish levels below which uptake experiments were conducted. The TL_m, the time at which 50% of the test animals are killed (Henderson and Tarzwell, 1957), was used as a measure of toxicity. Continuous-flow experiments were conducted at 14 C for 96 hr in water of constant mercury concentrations as provided by a proportional diluter of the type described by Mount and Brungs (1967). Groups of one hundred adult D. pulex and ten S. gairdneri were exposed to one of five mercury concentrations ranging from 5 - 100 ng Hg/ml (Fig. IIa) and 10 - 200 ng Hg/ml (Fig. IIb) respectively. Animals were periodically checked for mortality and dead ones removed.

Fig. IIa. The effect of mercury concentration on the survival of Daphnia pulex. Continuous-flow experiments conducted at 14 C for 96 hr.

O——O Hg as CH_3HgCl

△——△ Hg as $HgCl_2$

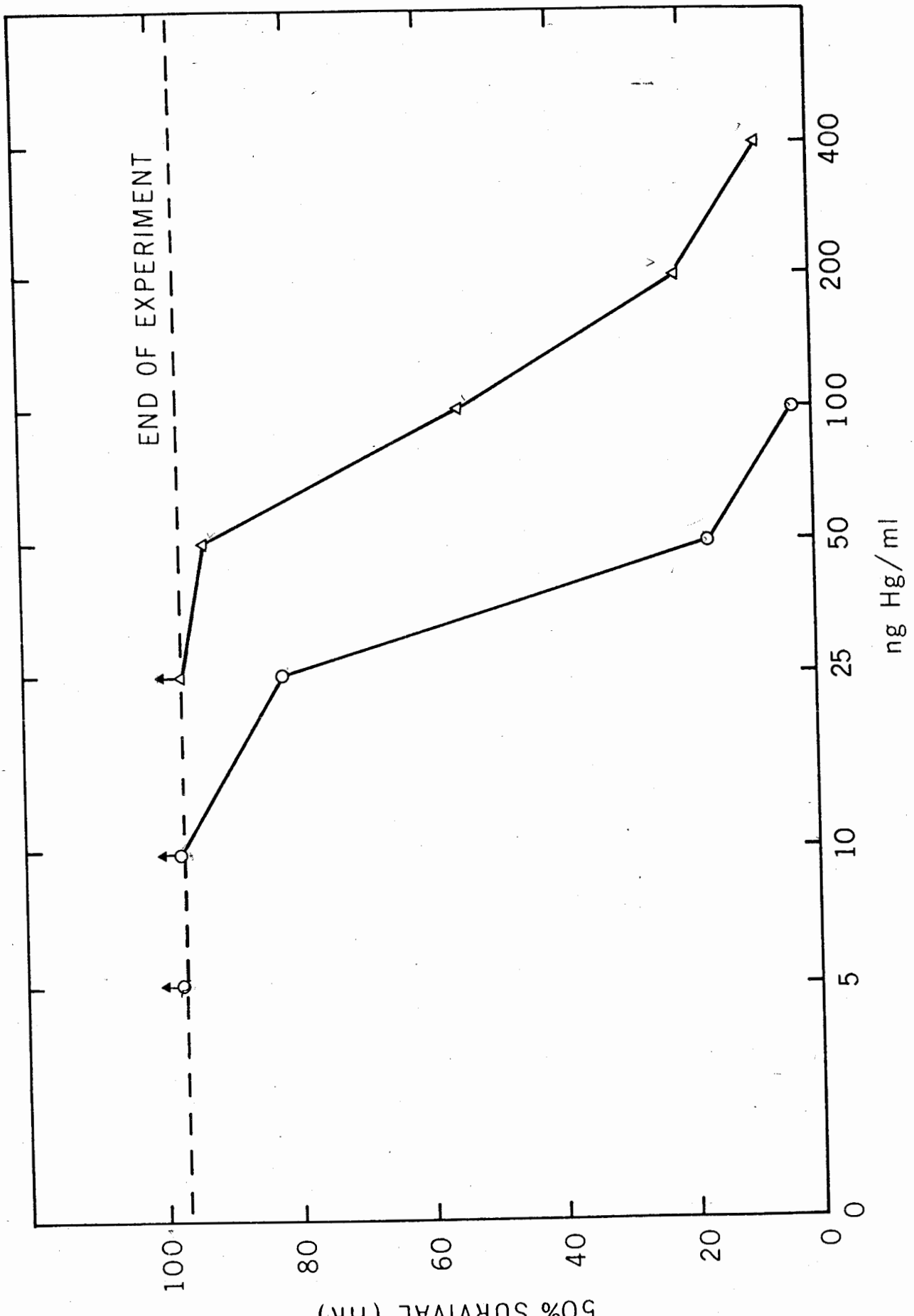
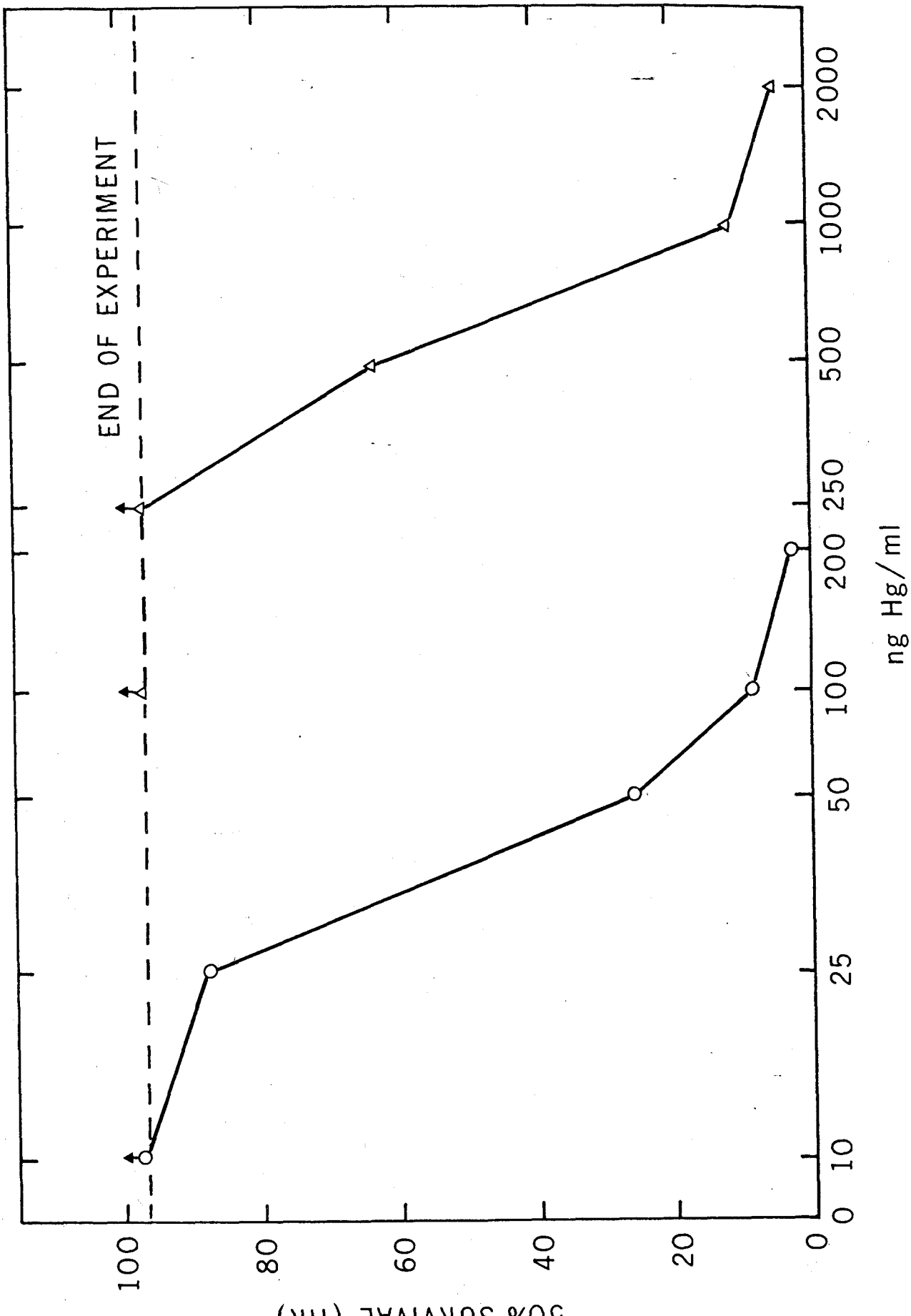

END OF EXPERIMENT
50% SURVIVAL (HR)
0
20
40
60
80
100
0
5
10
25
50
100
200
400
ng Hg/ml

Fig. IIb. The effect of mercury concentration on the survival of Salmo gairdneri. Continuous-flow experiments conducted at 14 C for 96 hr.

O——O Hg as CH_3HgCl

Δ——Δ Hg as $HgCl_2$


END OF EXPERIMENT
50% SURVIVAL (HR)
0
20
40
60
80
100
0
10
25
50
100
200
250
500
1000
2000
ng Hg/ml

Appendix III. Determination of percentage withdrawal of methylmercury from the water by Salmo gairdneri.

To determine the percentage withdrawal of methylmercury from the water, it was first necessary to calculate the ventilation volume (the volume of water passing over the gills). Ventilation volume values in the literature for Salmo gairdneri were plotted against weight and a regression line calculated. The significant linear relationship indicated a ventilation volume of 12.8 ml/min for an 8 g trout (Fig. III). Since the slope of the line is critical at low ventilation volume and a slight change could double or halve the volume, this value was compared with a calculated ventilation volume based on oxygen uptake of an 8.47 g sockeye salmon as reported by Brett (1965). This size of fish approximated the mean weight (8.12 g) of the trout used in my experiments. Ventilation volume was calculated as follows:

$$V_G = \frac{V_{O_2}}{(P_{IO_2} - P_{EO_2})aW_{O_2}}$$

where V_G = Ventilation Volume

V_{O_2} = Oxygen Uptake (ml O_2/min)

P_{IO_2} = Inspired Oxygen Tension

P_{EO_2} = Expired Oxygen Tension

aW_{O_2} = Oxygen Solubility Coefficient in Water

(ml O_2/ml H_2O/mm Hg at test temperature)

Fig. III. The relationship between ventilation volume and weight of Salmo gairdneri derived from published values. Ventilation volume was related to weight by the following linear equation, $Y = 0.147X + 12.2$; $r = 0.97$.

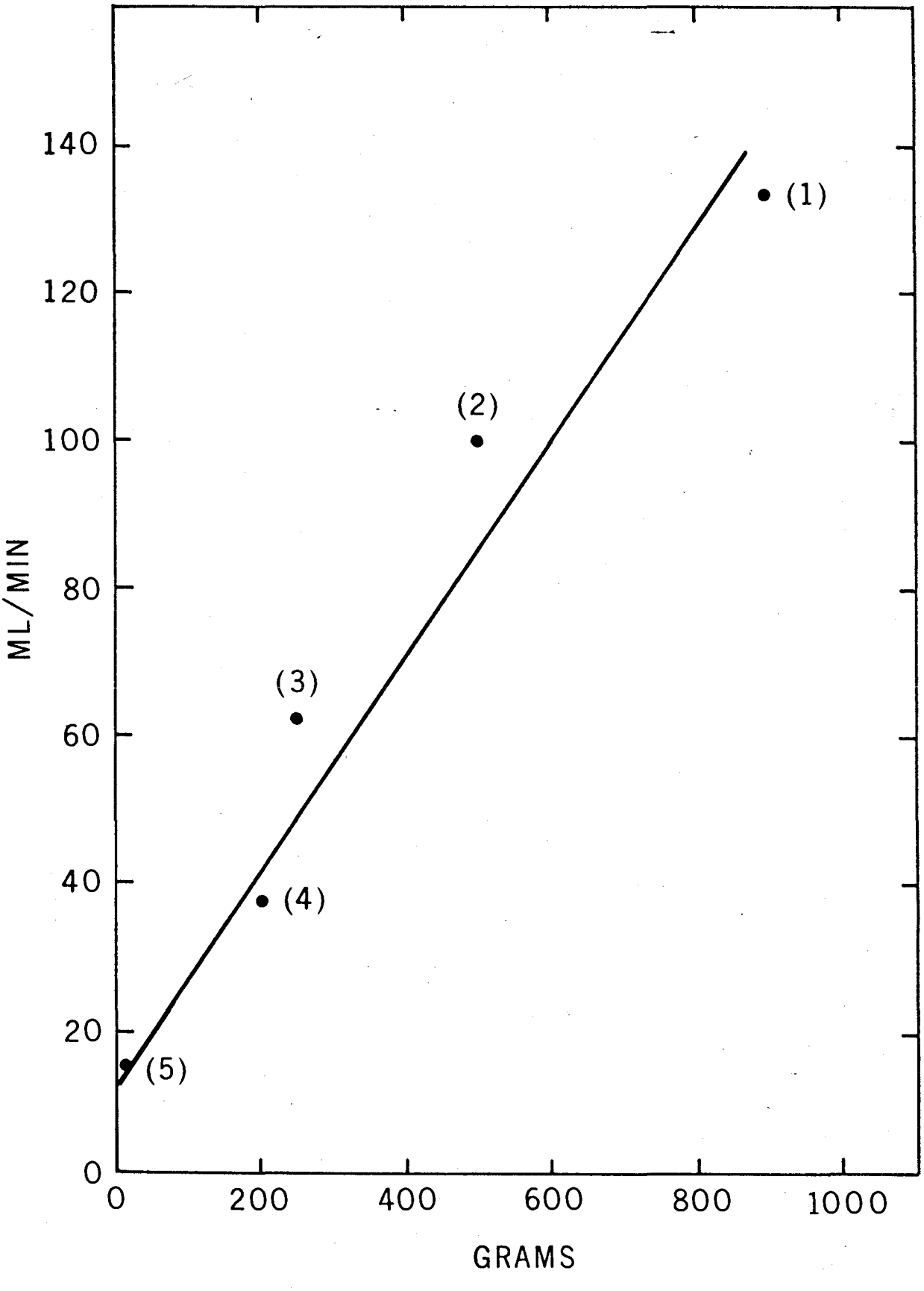
(1) Van Dam (1938)

(2) Hughes and Saunders (1970)

(3) Holeton and Randall (1967)

(4) Davis and Cameron (1970)

(5) Lloyd (1961)


140
120
100
80
60
40
20
0
ML/MIN
0
200
400
600
800
1000
GRAMS
(1)
(2)
(3)
(4)
(5)

Brett calculated an oxygen uptake for a 8.47 g sockeye at one-quarter activity (presumed equivalent to test conditions in this experiment) as 1.57 mg O_2/kg/hr (1.099 ml O_2/l or 0.018 ml O_2/min). At 13 C, $aWO_2 = 4.68 \times 10^{-5}$ (Randall, 1970). Assuming an inspired oxygen tension of 155 mm Hg (760 mm x 0.209(water vapor pressure at 13 C)) and that 40% of available oxygen is utilized (J.Davis, personal communication),

$$V_G = \frac{0.018}{(155 - 101.75) (4.68 \times 10^{-5})} = 7.18 \text{ ml/min}$$

As this value was about 60% of that derived from the regression line, I assumed an average value of 10 ml/min as a ventilation volume in my calculations.

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