RNA (Table 2) in the protoplast assay. The proportions of plaques seen at 41°C (Table 3, column 10) is not significantly different from the expected 1 to 2 percent of the numbers developing at 34°C. Thus the ts phenotype of the initiating ts-$Q\beta$ is faithfully inherited. Column 11 gives the number of ts-infectious units per reaction mixture calculated from the dilution used; column 12 lists the corresponding cumulative sums. No evidence of the synthesis of infectious RNA which could produce plaques at either 34° or 41° C appeared in the control nonprimed reaction. The corresponding negative columns are therefore omitted from Table 3.

The average infective efficiency of the RNA in the protoplast assay is 2×10^{-7}. The initial input in tube 1 was 0.2 μg corresponding to 1.2×10^{11} strands and 2.4×10^{4} plaque forming units. Since each transfer involves a 1 to 10 dilution, it is clear that less than one of the 1.87×10^{5} plaque formers observed in the 5th tube can be ascribed to the initiating ts-$Q\beta$-RNA. Finally, by tube 7 which contains 3.6×10^{12} new strands, the number of plaque formers (1.8×10^{5}) exceeds in absolute terms the number (1.2×10^{4}) of old strands present. It is clear that the serial dilution experiment has demonstrated the appearance of newly synthesized infectious RNA possessing the temperature-sensitive phenotype.

In the lower portion of Fig. 2 the outcome of the experiment shown in Table 3 is summarized by plotting against time the cumulative sums of the RNA synthesized (column 6) and plaque formers at 34°C (column 12). The fact that the plaque formers at 41°C are not statistically above the background of the assay of ts-$Q\beta$-RNA means that no detectable wild type $Q\beta$-RNA has been produced, a fact indicated by the open squares. For comparison the control reaction of Table 3, in which the initiating RNA was omitted, is similarly plotted on the same scale in the upper part of Fig. 2. No significant synthesis of either RNA or infectious units were observed.

It is apparent from the experiments described that one and the same normal replicase can produce distinguishably different but genetically related RNA molecules. The genetic type produced is completely determined by the RNA used to start the reaction and is always identical to it. The following two conclusions would appear to be inescapable from these findings; (i) the RNA is the instructive agent in the replicating process and therefore satisfies the operational definition of a self-duplicating entity; (ii) it is not some cryptic contaminant of the enzyme but rather the input RNA which multiplies.

The experiments described generate an opportunity for studying the genetics and evolution of a self-replicating nucleic acid molecule in a simple and chemically controllable medium. Of particular interest is the fact that such studies can be carried out under conditions in which the only demand made on the molecules is that they multiply; they can be liberated from all secondary requirements (for example, coding for coat protein, and so forth) which serve only the needs and purposes of the complete organism.

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Copper and the Role of Isopods in Degradation of Organic Matter

Abstract. *On an artificial substrate of filter paper,* Porcellio scaber *cannot extract copper from leaf litter. If one increases the copper content of the food by soaking the leaves in solutions of $CuSO_4$ or in organic extracts, assimilation of copper becomes possible, but only at concentrations higher than 1 microgram of copper per milligram of ash. This is too high a level for primary vegetable matter to be considered a plausible source of copper for isopods. I present evidence that in fecal material the critical level at which assimilation of copper becomes feasible is lower than in primary organic material by nearly an order of magnitude, and that isopods are obliged to switch to coprophagy in order to allow accumulation of copper in their bodies.*

Terrestrial isopods constitute an important link in the feeding chain that connects plant material with the humus of fertile soils. So far they have been considered to be primary consumers, breaking down the original plant material and thus preparing it for attack by other types of consumers such as mites, collemboles, protozoans, and microorganisms (*1*). In keeping with this picture it has been held that isopods consume large amounts of litter and other plant material, but cause only small alteration in the chemical composition of their food.

However, these animals have very considerable quantities of calcium in their integument (*2*) and of copper in their hepatopancreas (*3*). They lose calcium during each molt and copper when they feed on what has been considered to be their staple diet (*4*); thus they must compensate the loss of the two elements. Calcium can be extracted from the food rather efficiently (*2*); moreover, these animals often reingest their exuvia after molting. I now deal with copper.

I investigated several populations of *Porcellio scaber* L. from the outskirts of Vienna, Austria. Their standard food came from a large and thoroughly mixed batch of leaf litter from *Populus nigra*, collected in spring (after outdoor wintering) and stored at room temperature; its copper content was 0.13 to 0.32 ($X = 0.24$) μg Cu per milligram of ash. Before feeding experiments the leaves were dried in a desiccator for 24 hours, weighed, soaked in various extracts (to be specified), dried, weighed again, and then offered to single animals, each in a small petri dish with a disk of moist filter paper on the bottom.

Each experiment, lasting 5 days, was performed at 20°C in natural light.

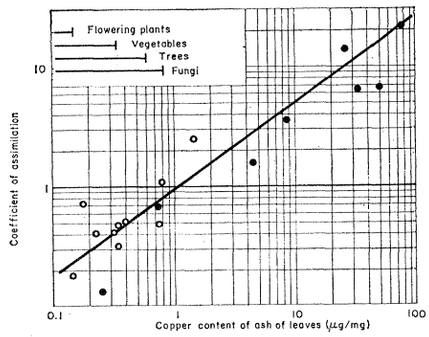

Fig. 1. Log-log plot showing relation between copper content of food and assimilation of copper by *P. scaber*. Assimilation is given as copper input:output; a coefficient of 1 signifies a steady-state level at which as much copper is lost as gained. Solid circles: leaves soaked in solutions of $CuSO_4$ at 0.002 (the highest four points), 0.0002 (the two medium points), and 0.00002*M* (the lowest two points). Open circles: leaves soaked in extracts of fecal substrates (see text). Boiled extracts give slightly higher copper values than fresh extracts. Inset (from *6*) shows upper limits of copper contents for main groups of primary plant material.

After each experiment, leaves and feces were dried in a desiccator, weighed, and ashed at 550°C before their copper contents were determined by chelation with zinc dibenzyldithiocarbamate (*5*). In this way feeding budgets, changes in relative contents of ash and organic matter, and copper budgets for individual animals could be established during short-term experiments. Results of the main set of experiments appear in Table 1.

It had been found (*4*) that under such conditions isopods eat too much relative to their energy requirements for growth and maintenance. There is probably a direct connection between overfeeding and the fact that under such conditions *P. scaber* consistently lose copper through their feces. Such a connection would be a severe handicap for animals that are supposed to live on a diet of leaf litter. Indeed, if one prevents the accumulation of feces in the experimental dish, terrestrial isopods do not survive for many weeks if they are fed leaf litter exclusively; whereas, in a culture dish having a rich substrate of feces, organic material, and microorganisms, the same diet suffices to maintain them for many generations.

In order to determine whether the animals were inherently incapable of assimilating copper under such experimental conditions, or whether the availability of copper was too low, leaves were soaked for 24 hours in 0.00002, 0.0002, and 0.002*M* solutions of $CuSO_4$, as well as in fresh and boiled extracts from the substrate that had accumulated for many months or years in large vessels housing *P. scaber*. Figure 1 shows clearcut proportionality between copper content of food and assimilation of copper [expressed as the quotient (copper content of leaves)/(copper content of feces), both input and output being given as micrograms of copper per animal per 1000 hours]; it also shows that the steady state, in which copper is neither lost nor gained, is achieved at a copper concentration in the food of not less than ~ 1 μg per milligram of ash—greater than the copper content of almost any vegetable matter that *P. scaber* could use as food (Fig. 1 inset, from *6*). Thus, under such conditions, feeding exclusively on primary vegetable matter, *P. scaber* could not have accumulated the large copper stores that so characterize it and other species of terrestrial isopods (*3*).

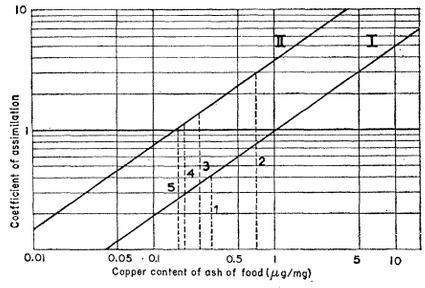

Fig. 2. Log-log plot illustrating the mechanism of copper extraction from food by *P. scaber*. The basic assumption is that the relation between total copper content of food and coefficient of assimilation for primary plant material (regression line *I*) differs from that for fecal organic matter (regression line *II*), the difference being in the level at which extraction of copper from food becomes possible—not in the slope. Dashed ordinates with numbers illustrate copper assimilation during hypothetical feeding cycle, starting with leaf litter containing copper at 0.25 μg per milligram of ash (*1*) and then switching to coprophagy (*2* to *5*).

In order to maintain copper levels in its body, *P. scaber* could revert to coprophagy after feeding on litter; the feces would contain the copper lost during the digestion of litter. Coprophagous feeding by these animals has been proven (*4*). If one assumes that the relation between Cu input and Cu output found for a litter diet holds equally for a fecal diet, it follows that in a well-digested fecal substrate the copper content would approach the steady-state level of about 1 μg Cu per milligram of ash of feces. Accumulation of copper beyond this value would cause it to be assimilated by the animals; extraction of the metal to less than the critical value would cause the animals to lose more copper from their stores in the hepatopancreas. Under such circumstances *P. scaber* could maintain its

Table 1. Food and copper budgets of *Porcellio scaber* fed leaves soaked in solutions of various copper contents.

P. scaber		Feeding budget per animal, dry substance (μg/hour)		Ash content (% dry wt)		Copper				
						In ash (μg/mg)		Budget per animal (μg/1000 hr)		Coefficient of assimilation (input:output)
No.	Weight (mg)	Input	Output	Leaves	Feces	Leaves	Feces	Input	Output	
1*	73	158	115	17.5	24.5	0.18	0.24	4.9	6.8	0.72
2*	80	32	13.6	19.5	20.0	26	4	150	10.9	13.7
3	90	68	48	17.5	31.0	0.15	0.68	1.8	10	0.18
4	72	43	39	18.0	32.0	.8	.43	6	5.5	1.1
5	71	178	140	19.0	23.0	.23	.6	7.8	19.5	0.4
6*	75	17	24	20.0	19.5	48.8	3.14	110	16.2	6.8
7*	48	48	41	21.0	22.5	8.7	2.6	87	24	3.6
8*	65	71	68	15.5	19.5	0.26	1.26	2.8	21.2	0.13
9*	95	81	73	17.4	21.5	1.47	0.52	20.2	8	2.5
10*	53	85	31	17.5	36.0	0.33	1.07	5	11.8	0.42
11	57	100	74	17.5	27.0	.41	0.72	7.2	14.5	.5
12	82	100	60.5	16.0	26.0	.35	.75	5.6	11.8	.48
13*	50	36	18.5	18.8	18.0	77.8	7.2	525	24	21.8
14*	43	75	32.5	19.0	31.0	4.45	4.3	64	41.5	1.55
15*	50	125	92	17.5	21.5	0.75	1.2	16.4	24	0.68
16*	82	34	23	20.0	18.5	.75	2.21	5.1	10	.5
17	43	105	59	18.5	31.0	.35	1.14	6.8	21	.32
18*	62	86	38	13.2	38.5	33.4	4.0	375	59	6.4

* Mean values from two experiments; all other values are individual.

Table 2. Two series of measurements of copper contents of ashed substrates.

Sample	Copper content, ash (μg/mg)	
	Series I	Series II
1	0.093	0.28
2	.073	.20
3	.088	.16
4	.010	.20
5	.074	.19
6		.30
$\bar{x}$	.086	.22

copper holdings only in a rather precarious manner, since the original source of the metal (the leaf litter) contains only about 25 percent of the concentration required to merely compensate the loss of copper by digestion.

Realization that the copper content of a well-digested fecal substrate should approach the steady-state of copper assimilation enables one to determine whether the assumption holds that assimilation of fecal copper follows the same rules as assimilation of leaf-litter copper. This I did by analyzing the copper content of substrates in culture dishes in which large populations of *P. scaber* had been fed for years entirely on carrots and poplar-leaf litter; the substrates consisted almost exclusively of accumulated droppings. I made two series of measurements from various dishes and at different times of year (Table 2).

The values of Table 2 represent copper levels lying between the concentration in the feces originally produced by *P. scaber* (after feeding on primary vegetable matter) and some unknown steady-state level. Since according to Fig. 1 the feces produced by *P. scaber*, after feeding on leaves containing copper at about 0.24 μg per milligram of ash, would contain approximately 0.7 μg Cu per milligram of ash, the expected steady state could be as low as or lower than 0.086 μg Cu per milligram of ash, the lower of the mean values in Table 2. But if one allows for individual or seasonal differences, a steady-state level of 0.15 μg is assumed to hold for assimilation from fecal material.

Thus, by reverting to coprophagy, *P. scaber* should be able to solve its problem of copper retrieval. A copper content in the new food of 0.015 percent—well below that of the original source of copper—would suffice to maintain its body concentration of copper, whereas under the conditions holding for digestion of leaf litter, the animals would have lost copper heavily at this concentration. An explanation of this new relation is afforded by the activity of microorganisms, which most likely convert so much of the copper bound to proteins into less-tightly bound forms that a larger percentage of total copper can be assimilated by the isopods. Copper in living material can exist in both easily dissociable and tightly bound states (*7*).

Figure 2 summarizes the new concept of feeding activity by *P. scaber*. It is assumed that a population of isopods starts feeding on leaf litter, from which copper can be extracted only if the copper concentration is around 1 μg per milligram of ash (Fig. 2: regression line *I*). Starting with poplar litter containing 0.25 μg (Fig. 2: *1*), the animals would lose copper heavily and the first batch of feces would contain $\sim$ 0.7 μg Cu per milligram of ash. After the onset of microbial activity the isopods would switch to coprophagy, for which the steady-state level is assumed to be $\sim$ 0.15 μg Cu per milligram of ash (Fig. 2: regression line *II*). Thus the starting value of feces copper (Fig. 2: *2*) would allow very high retention of this element under the new conditions. Within three more cyclings of the feces (Fig. 2: *3* to *5*) the copper level in the fecal substrate would approach the steady-state level. By alternating between consumption of primary vegetable matter and coprophagy, *P. scaber* could fill its demand for copper and perhaps for other essential food components.

This interpretation has two corollaries: isopods probably consume less primary vegetable matter and are more implicated in secondary breakdown of organic material than has been assumed.

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Nitrogen- and Helium-Induced Anoxia: Different Lethal Effects on Rye Seeds

Abstract. *Prolonged exposures to acute anoxia caused progressive reductions in the viability of hydrated seeds of Prolific rye. For equal exposures of 9 days or longer, mortality was significantly higher in helium than in nitrogen. The findings suggest that prolonged use of helium as a component of atmospheres in manned space capsules may be harmful.*

Atmospheres within manned space capsules launched by the United States have so far consisted of 100-percent oxygen at 0.34 atm. There is general agreement among space scientists that atmospheres consisting of gas mixtures are desirable, possibly essential, for prolonged missions in space. Difference of opinion exists regarding choice of a second gas—should helium replace nitrogen. Saving in weight, higher thermal conductivity, and reduced probability of embolism have been cited as advantages of helium. The opposite view is that biologic effects of helium are insufficiently understood and that its unknown physiological hazards may exceed its putative advantages (*1*).

Despite their chemically unreactive nature, noble gases can produce various responses in biologic systems (*2*). Although their mechanisms of action are unknown, their effects must reflect their physical properties. Magnitudes of response can be correlated with their molecular weights whether they are used to dilute oxygen (*3*) or as pure anoxic environments (*4*). Unfortunately, current knowledge of the biologic effects of rare gases cannot resolve the arguments concerning the relative merits of nitrogen and helium as components of gas mixtures.

Recent findings regarding effects of these gases on germinating seeds seem especially pertinent. Seeds of Prolific spring rye were hydrated anaerobically and held in anoxia for up to 15 days. Dry, resting seeds (50 per dish) were placed in petri dishes lined with moist filter paper. Samples, 100 to 200 seeds per treatment, were promptly sealed in helium-tight chambers of welded Lucite which were quickly evacuated to −76 cm-Hg and then filled and flushed for several minutes with nitrogen or helium. The chambers were thereafter kept in