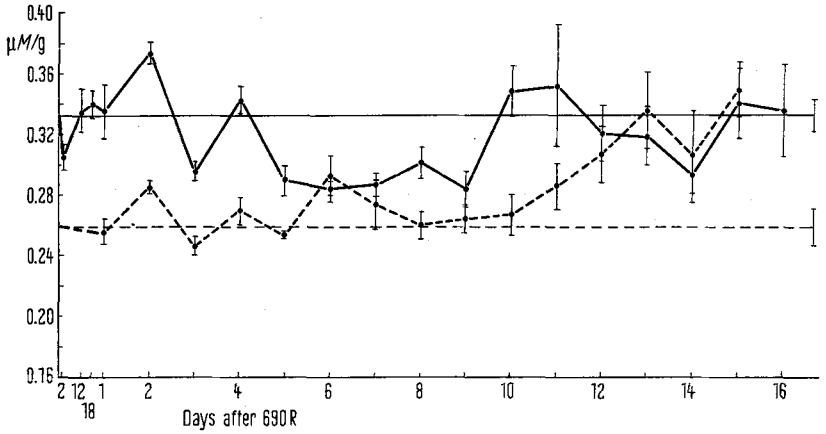

Citrate in mouse liver after 690 R. ·——· fed mice, ·– – – – · mice fasted for 24 h before killing. 8 livers for each point, standard deviations.

After whole body X-irradiation with 690 R, the content was decreased somewhat on certain days after irradiation in fed mice (see Figure). However, this change depends to a high degree on the irregular food intake after irradiation. To single out the effect of irradiation from the influence of irregular food intake, in a second series the citrate content was measured in the liver of mice fasted for 24 h before being killed. At the time of killing, all mice were therefore in the same nutritional state.

Under these circumstances, the content of citrate was almost unchanged for 11 days after irradiation, followed by an increase on 12th–15th day up to the value of normally fed mice (dotted line in the Figure).

Discussion. The results show that the citrate content is scarcely influenced in mouse liver by the irradiation and citrate synthesis via the citratesynthetase reaction seems to be normal even after lethal X-ray doses. Changes in fed mice depends mainly on starvation effects following irradiation, since the citrate content remains almost unchanged in fasted mice over a period of 11 days after the exposure (see Figure). Therefore, neither the inhibition of phosphofructokinase nor the activation of acetyl-CoA-carboxylase by citrate is considered to be altered during this period after irradiation. It is improbable that on the first to eleventh days after irradiation any irradiation induced modifications of glycolysis or fatty acid synthesis are effected by citrate. However, the elevated citrate level in starved mice on the 12th–15th day might influence somewhat the activities of these 2 enzymes in vivo. It is difficult to decide whether the extramitochondrial acetyl-CoA content is influenced by the change in the level of citrate, because acetyl-CoA can come from anaerobic glycolysis as well as from degradation of fatty acids. Hence, the changes in acetyl-CoA content after irradiation[10] are different from those of citrate[11].

Zusammenfassung. Nach einer Bestrahlung mit 690 R beruhen Veränderungen des Citratgehaltes in der Leber gefütterter Mäuse auf unregelmässiger Nahrungsaufnahme, während in 24 h hungernden Mäusen der Citratgehalt nach 690 R über 11 Tage fast unverändert bleibt und erst vom 12.–15. Tag nach der Bestrahlung ansteigt. Die Ergebnisse werden diskutiert im Hinblick auf Phosphofruktokinase – und Acetyl-CoA-Carboxylase-Aktivität.

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Possible Abiotic Origin of Precambrian Microfossils

Biochemical and electron microscope investigations of selected precambrian rocks have shown evidence for the existence of microfossils believed to be contemporary with the rocks[1,2]. Related findings have revealed indigenous amino acids and alkanes in the samples.

Over the past decade it has been shown that a large variety of biochemicals can be formed from simple gases and liquids under the action of high energy sources using hypothetical primitive earth conditions (for reviews see reference[3]). Recent results have also demonstrated that the transition from simple molecules to macromolecules is often accompanied by a separation of microstructures from the medium[4–6]. These findings and those to be presented here support the suggestion of a possible abiotic origin of the microfossil forms found in the precambrian rocks.

As a starting material for our experiments we used ammonium thiocyanate (NH_4SCN) which is a known product of juvenile volcanic gases[7] and has been produced under simulated primitive earth conditions[8]. In previous publications we showed that a small amount of methionine is synthesized by UV-irradiation of NH_4SCN[9] and that cell-like structures are produced in the presence of formaldehyde[10]. In the present communication we report evidence demonstrating a resemblance between these abiotically produced microspheres and the microfossils on the basis of (a) morphology, (b) chemical composition and (c) physical properties.

We irradiated aqueous solutions of NH_4SCN (0.01–2 *M*) with a submerged Pen-Ray quartz lamp (Ultraviolet Products SC-1) for various times up to 315 min. All reaction solutions were first filtered with a 0.22 μ Millipore filter. After a few minutes of irradiation, the solutions showed a white turbidity. We placed a drop of the products on a slide and examined it with the light microscope. The size varied up to 10 μ in diameter and was an increasing function of initial reactant concentration and irradiation time.

About 10 min after the radiation had ceased (and a heavy white suspension was obtained), the microspheres began to form aggregates of larger spheres and chains. After standing for 2 h, the size of the aggregates continued to increase (up to 200 μ) and fibrillar aggregates were observed (Figure 1). After 100 h, nearly all the microspheres had formed large aggregates. Higher concentrations and aging increased the aggregating tendencies. Our observations are similar to those reported by other workers investigating the behavior of polypeptides precipitated from solution[11]. In order to see how the microspheres would behave in the presence of adenosinetriphosphate (ATP), which has been synthesized abiotically[12], we added microsphere suspensions to 0.001 *M* ATP solutions. The aggregation proceeded more rapidly and there was more surface contact between the microspheres forming the aggregates than previously. Figure 2a and c show electron micrographs of these aggregates.

The resemblance of the microsphere aggregates to precambrian microfossils is shown in Figure 2. The interior structure and dimensions appear to be similar. Since all reactant solutions were sterilized by filtration, irradiated with UV-light, and kept in closed tubes, and since these forms appeared consistently, the possibility of non-specific contamination is effectively ruled out.

Since our microstructures resembled microfossils we next investigated their stability. In tests for solubility, the microspheres were found to be insoluble in water, dilute HCl, dilute KOH, methanol, propanol, and benzene.

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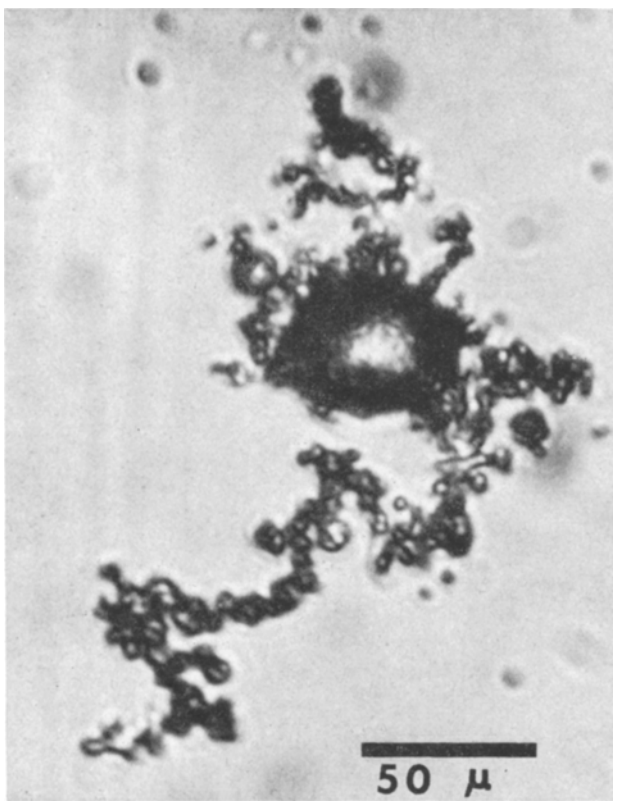
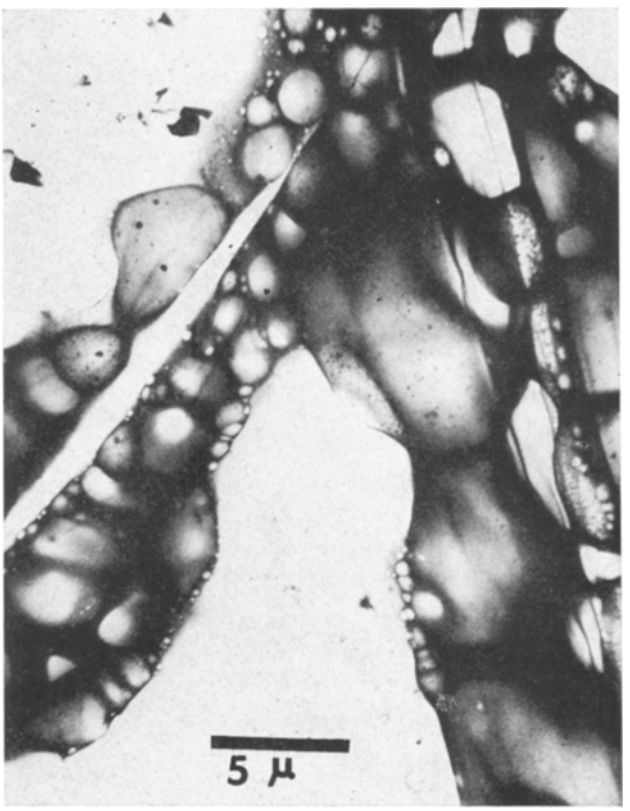
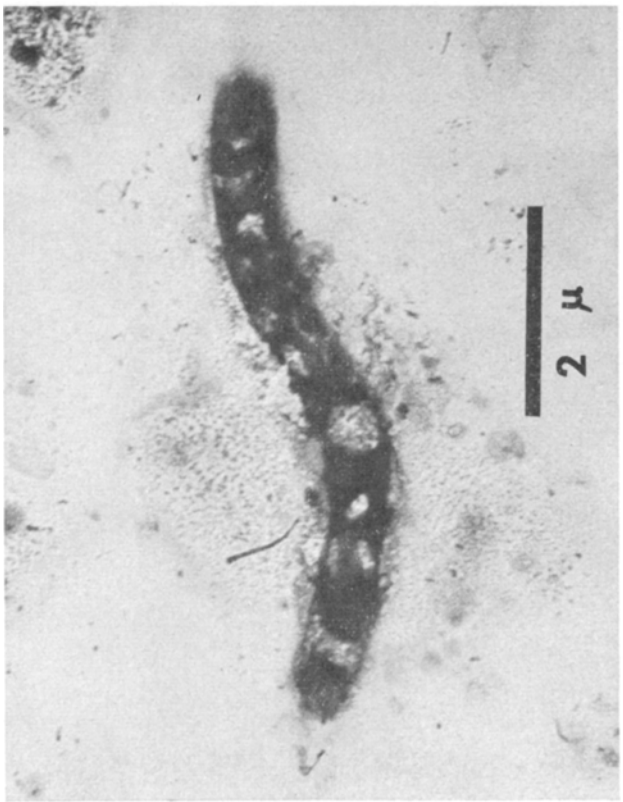


Fig. 1. Aggregates of microspheres in the form of a large sphere and chain.

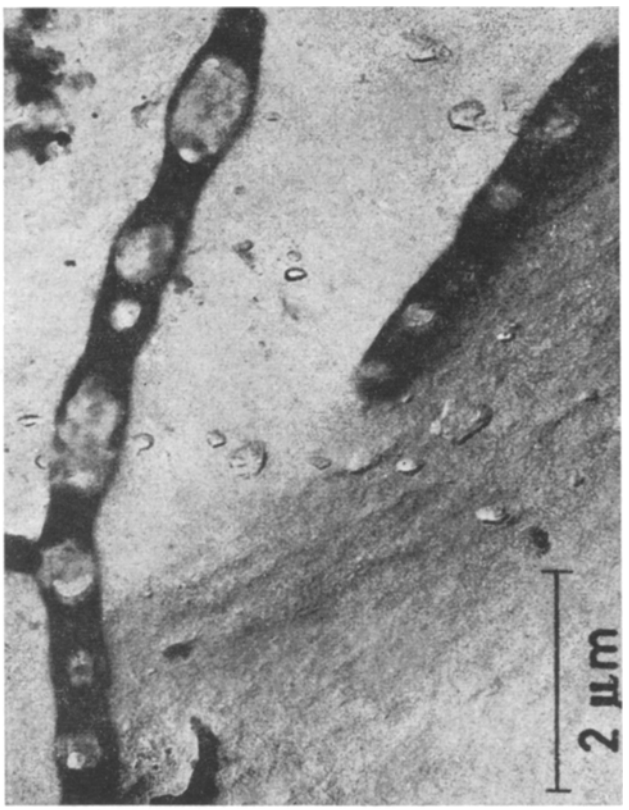


a

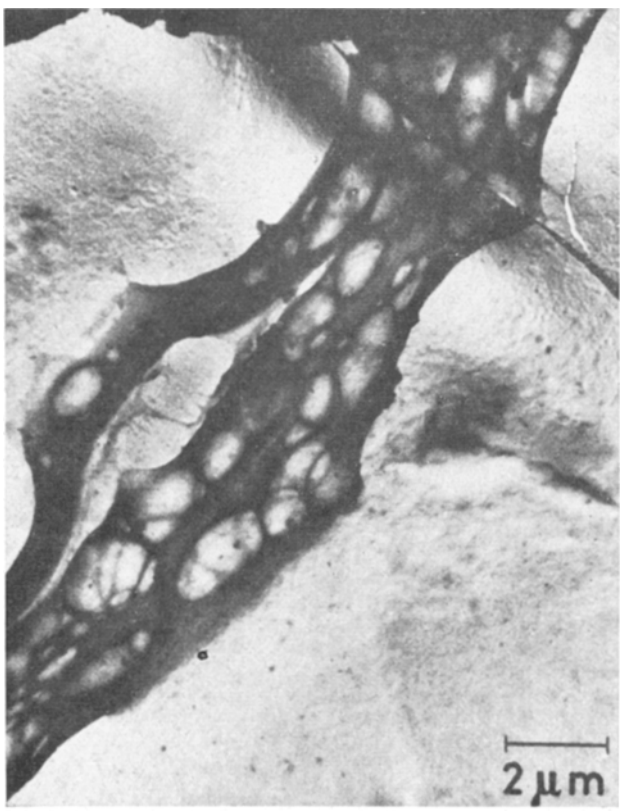
Fig. 2. Morphological similarity between the microsphere aggregates (a, c) and the precambrian microfossils (b, d). The microfossil photographs are from[2].



b



d



c

We placed a drop of the product suspension on a microscope slide and allowed it to dry. Then water was added to the dried matrix. The microspheres appeared to be unaffected by this process which was carried out several times on the same drop. We also placed a drop of microsphere suspension on a slide placed in an oven at 100 °C for 1 h, and rehydrated it afterwards. Another drop was held at − 20 °C for 1 h. In both cases, the microspheres appeared to be unaffected with regard to gross morphology.

It seems that under common natural conditions, the microspheres maintain their integrity and thus could easily have become fossilized. Their stability with respect to heat and drying shows that their survival would be quite plausible.

Studies have been reported concerning the biochemical composition of ancient rocks which contain microfossils. To consider the possible relation of this to our own investigation, we placed 0.264 m*M* NH_4SCN in 5 ml H_2O irradiated with a PenRay lamp for various times under a nitrogen atmosphere in an ice water bath. The product was hydrolyzed in 6*N* HCl under nitrogen for 20 h at 105 °C. Then it was evaporated, dissolved in H_2O, and $AgNO_3$ was added until no further precipitate appeared. The supernatant was collected and dilute HCl was added until no more precipitate was formed. The final supernatant was collected, evaporated to dryness, and analyzed on a Technicon Amino Acid Analyzer (Table). These results may be compared to those of SCHOPF et al.[1] who considered the amino acid composition of selected precambrian rocks.

ABELSON[13] has found that glycine and alanine have comparable degradation rates. With this in mind, we considered the amino acid content of several bacterial

and viral proteins for their glycine/alanine ratio[14]. When taken together, the overall glycine-to-alanine ratio of cytochrome C (*Neurospora*), ferridoxin (*Clostridium*), tobacco mosaic virus, ribonuclease (*Aspergillus*), tryptophan synthesase (*E. coli*), and azurin (*Pseudomonas*) is 1.0. This ratio may be compared to the reported ratio of 60 for precambrian samples. The large difference between contemporary organisms and the microfossils cannot be explained by a difference in amino acid degradation rates. It is of interest to note that our synthetic (abiotic) glycine/alanine ratio of 14 (Table) is significantly closer to the fossil ratio.

The molecular mechanism involved in the formation of the complex thiocyanate structures described here is unclear. However, we have demonstrated that under plausible primitive earth conditions such formation is a spontaneous occurrence. Furthermore, we have observed morphology, chemical composition, and stability also found in geological microfossils. These results suggest that the microfossils may actually be remnants of cell precursors or that the earliest cells possessed a biochemistry significantly different from that found in present day organisms. In any case, the need for more careful evaluation of the significance of these microfossils is clearly pointed out[15].

Amino acid analysis of irradiated NH_4SCN microspheres following hydrolysis

Time of irradiation (min)	Glycine μM/mM NH_4SCN	Alanine μM/mM NH_4SCN	Glycine / alanine
75	0.14	0	–
195	3.22	0.23	14
315	0.38	0.17	2.23

Résumé. Les microsphères obtenues par irradiation UV du thiocyanate d'ammonium sont comparées du point de vue morphologique, chimique et de certaines propriétés physiques, à certains microfossiles précambriens.

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Axo-Somatic Synapses in Procerebrum of Gastropoda

Central synapses of Gastropode molluscs are generally believed to be of axo-axonic type[1,2]. In fact no axo-somatic synapses have been found electron-microscopically in ganglia of different Gastropode species[3–6]. In one case a synapse-like profile was described in trophospongium of cerebral ganglia of Glossodoris[7]. Even if this was an axo-somatic synapse, it was a very unusual picture for this tissue, where synapses were reported to be mainly axo-axonic type[7]. So it is generally accepted that in Gastropodes the bodies of nerve cells do not play any role in the synaptic transmission, since the site of interneuronic contacts are restricted to the centrally located neuropile.

However, such organization is not a general rule: we were able to demonstrate in the procerebrum of the Pulmonates *Helix* and *Limax* abundant structures that were typical axo-somatic synapses so far as their morphology is concerned.

Procerebra of *Helix pomatia* and of *Limax cinerea-niger* were fixed in 1.5% OsO_4 buffered with collidine, embedded in Durcupan ACM, cut on the LKB Ultrotom III. Sections were contrasted with lead citrate and examined in the Tesla BS 413A electron microscope.

Light microscopic studies have shown that the procerebrum differs in many respects from typical Gastropode ganglia: its neuropile has rather a lateral than central position and the cellular mass consists of very small (up to 10 μ in diameter) uniform, densely packed neurones[1]. The submicroscopic organization of procerebrum, a complete description of which we give elsewhere, is also peculiar in some respects. Axo-somatic synapses are one of these unusual features.

In both *Helix* and *Limax*, the presynaptic endings in the cellular mass of the procerebrum are of 2 types. The first type (Figure 1) contains dense-core vesicles of about 800–1200 Å in diameter, while the second type (Figure 2) contains clusters of empty vesicles with a diameter of 5–800 Å. The fibres giving origin to the first type endings, are usually thicker than those of the second type, though in some other respects there are similarities between them. Both types are varicose fibres consisting of thin segments and expansions with mitochondria and synaptic vesicles. Varicose expansions of both types are often contacted with more than 1 nerve cell, sometimes with 3–5 cells. Widening of synaptic cleft typical for mollusc

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