

4. Obtained from Horace Isbell, National Bureau of Standards.
5. Prepared by a modification of the method of P. R. Srinivason, D. B. Sprinson, and B. D. Davis, private communication. The weight ratio of shikimic acid-C¹⁴ added to the CTC subsequently formed was 0.55.
6. In the fermentations containing shikimic acid-C¹⁴ and in the pilot fermentations for these experiments containing unlabeled shikimic acid, the terminal concentrations of CTC formed were not increased over those formed in the control fermentation containing no added shikimic acid.
7. R. Kuhn and K. Drury, *Chem. Ber.* **84**, 563 (1951); B. L. Hutchings *et al.*, *J. Am. Chem. Soc.* **74**, 3710 (1952).
8. When added at zero hours, starch-C¹⁴ (uniformly labeled), D,L-lysine-2-C¹⁴, D,L-ornithine-2-C¹⁴, and glycine-2-C¹⁴ showed 10, 8.7, 4.2, and 12 percent incorporation, respectively.

27 December 1955

Shell Mineralogy in Paleozoic Invertebrates

Among the modern invertebrate groups that deposit shells of CaCO₃, some employ calcite exclusively, some employ aragonite exclusively, and still others combine discrete layers of each mineral. Several investigators have questioned the evolutionary stability of the mineralogic habit of compact groups, suggesting that there may have been change through time. But it has been possible to answer this question satisfactorily only for the more recent past, the late Mesozoic and Cenozoic. Rocks deposited during this interval have occasionally yielded shells that preserve unaltered their original mineralogy. Moreover, many of the organisms of this interval are so closely related to living forms that little change would be expected. As one attempts to follow groups backward in time, however, unaltered preservation becomes rarer and relationships to living forms more tenuous. Consequently, our answers become more suspect until, finally, among Paleozoic groups, only speculation has been possible.

Bøggild (1) put such speculation on a reasonable basis by assuming that the recrystallization of metastable aragonite to calcite destroyed the shell microarchitecture. With this assumption, he deduced the original mineralogy of the shells of many Paleozoic organisms. Unfortunately, the validity of the assumption could not be tested beyond the Mesozoic, for no Paleozoic fossils had been found that actually preserved the original mineralogy. Recently, however, two collections, one from the Buckhorn asphalt (Middle Pennsylvanian) in Oklahoma and the other from the Kendrick shale (Lower Pennsylvanian) of Kentucky, have yielded large faunas in which the preservation of intricate microarchitectural detail indicates retention of the original mineralogy. These collections appear to be the oldest yet found in

which the mineralogy is unaltered. Some preliminary observations of the mineralogy of several Paleozoic groups are reported here (2). In all cases in which direct comparison was possible, the mineralogy of these shells proved to be the same as that deduced by Bøggild. Therefore, his basic assumption is probably sound and his method applicable to most cases.

The study of the mineralogy of these shells proceeded as follows. In the case of the Kendrick shale material, the discriminations between calcite and aragonite were made with Leitmeier and Feigl's staining solution. The oil-soaked Buckhorn asphalt collections could not be cleaned sufficiently to permit reaction with this solution. Consequently, determinations were made instead by x-ray. Representatives of the following phyla were tested: Brachiopoda, Ectoprocta, Cnidaria, Mollusca, Echinodermata, and Arthropoda.

Brachiopods belonging to six genera were tested and found in each case to be composed exclusively of calcite. This finding supports the conclusion, derived from the calcitic mineralogy of living forms (3) and from the excellent preservation of most brachiopod fossils (1), that the group has always employed calcite. The genera tested, *Derbyia*, "*Chonetes*," *Marginifera*, *Linoproductus*, *Crurithyris*, and *Spirifer*, are distributed in two orders, the Strophomenida, which may have a few living representatives, and the Spiriferida, which has been extinct since the Jurassic.

One tetracoral, *Lophophyllidium profundum*, was discovered in the material and was also found to be composed exclusively of calcite. There has been much speculation about this group, because the living corals, Scleractinia, construct their skeletons of aragonite. The calcitic skeleton of *Lophophyllidium* shows that at least some of the tetracorals were calcitic. The general preservation of the group suggests that most, if not all, genera shared this characteristic.

Fragments of an unidentified Ectoproct bryozoan were recovered and proved to be composed exclusively of calcite, as are most living representatives of the group. The skeletal elements of at least one crinoid, including calyx cups, arm plates, and columnals, have been examined and are entirely calcitic, as are living crinoids and, in fact, all echinoderms. The pygidium of a single trilobite was recovered and, as Bøggild has indicated, was found to be composed entirely of calcite.

The collections contain a large number of gastropods which possess shells that combine discrete layers of calcite and discrete layers of aragonite. *Straparolus* (*Amphiscapha*) *sp.* and an unidentified euomphalaceid have an outer cal-

citic layer and an inner aragonitic one, thus giving some confirmation to Bøggild's conclusion that this mineralogy was characteristic for the group. One specimen of an unidentified bellerophon-taceid clearly shows a very thin calcitic outer layer and a massive inner aragonitic layer; but, in other individuals of the same species, the outer layer has not been recognized, and any conclusions regarding the mineralogy must be held in abeyance pending further investigation. Bøggild (1) has reported a bellerophon-taceid from the Ordovician which he considered to have been composed of calcite. It is possible that this group exhibits a temperature response in its mineralogy similar to the responses indicated for some recent mollusks by Lowenstam (3), although there may also have been an evolutionary change in mineralogy.

Among pelecypods, several forms show both calcitic and aragonitic layers. *Chaenocardia ovata* has an extremely thin outer layer of calcite—so thin, in fact, that it has been removed by abrasion over most of the shell—and a much thicker inner layer of aragonite. Other pelecypods that exhibit this characteristic are present but have not yet been placed even in major taxa.

A large number of pelecypods have shells consisting only of aragonite, but of them only *Leda* and *Astartella* have been identified. *Leda* is a nuculaceid, and, since living representatives of this group seem to be wholly aragonitic, it suggests little change in the mineralogy. A majority of the gastropods that were examined consist wholly of aragonite. Genera that have this mineralogy are *Sphaerodoma*, *Shansiella*, *Soloniscus*, and *Trepostira*.

A number of small, probably juvenile, pectenoids were found but could not be identified. Their shells appeared to consist wholly of calcite. In structure, their shells are unlike those of modern pectenoids, which have an aragonitic shell layer between layers of calcite or the structure of *Limipecten morsei* from the Kendrick shale, which Newell (4) believed to have a thin outer ostracum of calcite and a thicker inner ostracum of aragonite. Because of this lack of similarity and because the individuals observed may be juveniles, further investigation of the shell structure of pectenoids is necessary before any conclusions are reached.

A number of nautiloid cephalopods are present in both collections, and several unidentified shell fragments, as well as specimens of *Pseudorthoceras knoxense*, have been tested. The external shell has, in all cases, been found to consist entirely of aragonite, but tests of the cameral deposits of *P. knoxense* have consistently shown the presence of variable amounts of calcite. Thin sections of the cameral deposits show no recrystall-

ization, and it is concluded that the calcite is primary rather than secondary. No evidence was found that supported Bøggild's suggestion of the presence of calcitic layers in the outer shell, but sampling is, of course, quite inadequate to prove or disprove his contention. A goniatite, *Gastrioceras elkhornense*, was also tested and found to be composed of aragonite.

It is hoped that further study of this material will permit determination of the skeletal mineralogy of additional forms and the discovery of the micro-architectural characteristics of many groups. Preliminary tests of oxygen from the carbonate of some of these shells suggest that they will be suitable for O^{18}/O^{16} paleotemperature studies. It may be possible, therefore, to learn much of the temperature requirements and modes of growth of long-extinct forms.

FRANCIS G. STEHLI

California Institute of Technology,
Division of Geological Sciences,
Pasadena, California

References and Notes

1. O. B. Bøggild, *Kgl. Danske Videnskab. Selskabs. Skrifter, Naturvidenskab. math. Afdel.*, 9, 2, No. 2, 232 (1930).
2. Contribution No. 755 from the California Institute of Technology, Division of Geological Sciences.
3. H. A. Lowenstam, *Proc. Natl. Acad. Sci.* 40, 39 (1954).
4. N. D. Newell, *State Geol. Survey Kansas* 10, pt. I, 69 (1937).

20 December 1955

Radiation-Induced Fecal Fat Formation in the Rat

Studies on the effect of radiation on fecal fat thus far have not clearly distinguished between the effects of the radiation and the induced anorexia (1). Increases in fecal fat that were noted in human patients by Dodds and Webster (2), following x-ray therapy, were attributed to impaired lipid absorption induced by irradiation. Mead and coworkers (3), maintaining irradiated mice on fat-free diets, showed elevated fecal fats. These authors state that this fecal-fat increase was due to intestinal desquamation resulting from radiation injury. In contrast, Coniglio *et al.* (4), through fat-balance studies, have noted decreased fecal-fat after irradiation but have correlated such changes to the lessening of food intake during this period. To eliminate variation in fat excretion resulting from food intake, the studies reported here have dealt with fasted normal and irradiated rats. Further, since fecal lipid appears to be secreted by the intestinal wall (5), lipogenesis has been studied in

Table 1. Fecal fat in normal and irradiated rats.

No. of animals	No. of determinations	Dose (r)	Fecal fat (mg/g)	Specific activity (count/min mg)*	Total counts (count/min g)
64	31	0	56.4	1.3	75
34	17	1000	60.2		
39	21	1500	82.7	3.5	348

* Acetate-1-C¹⁴ was administered to 16 normal and seven irradiated animals. Specific activity and total count refer only to these animals.

two groups of animals by intraperitoneal injections of acetate-1-C¹⁴ (0.1 μ c) prior to the fecal collection period.

Female albino rats weighing 175 ± 15 g were given 1000- or 1500-r doses and fasted 48 hours while they were being kept in metabolic cages. The feces during this period were collected, pooled from two animals, and dried in a vacuum. Aliquots of fecal material were ground, and the total lipid was extracted. This latter process involved two incubations at 40°C with an alcohol-ether mixture (3:1) followed by a 12-hour ethyl ether Soxhlet extraction. The isolated lipids containing carbon-14 were analyzed by means of a nuclear-flow counter using a Berkeley scaler.

Fecal-fat excretion, as can be seen in Table 1, shows significant elevation with roentgen dose. Thus the feces from normal animals averaged a fat content of 56.4 mg/g and increased to 82.7 mg/g when a 1500-r dose had been administered. If taken separately, similar comparisons can be made in the groups injected with acetate-C¹⁴ and the non-injected groups. Here, irradiated animals receiving acetate showed a fecal-fat average of 99.0 mg/g as compared with 58.5 mg in the normals, while those receiving no acetate exhibited 79.2 and 55.2 mg/g under the same respective treatment.

Comparisons of lipogenesis have been made by noting the total counts found in the fecal fat from irradiated and non-irradiated animals. Such comparisons clearly show that the treated rats incorporated into the fecal lipids more than 4 times as much acetate-C¹⁴ as did the controls. In addition, the increase in specific activity from 1.3 in the controls to 3.5 in the irradiated animals would also be indicative of a stimulation in the fecal-fat synthesis.

Further studies to demonstrate the similarity in composition of the fecal fats from both groups were carried out by fractionating the isolated material into free fatty acids and mono-, di-, and triglycerides. This technique, as outlined by Mattson and Beck (6) showed that the lipid samples contained, on the average, 41 percent free fatty acids, 20 percent monoglycerides, and 39 percent di- and triglycerides from both normal and

x-rayed animals. Since increased lipogenesis has been demonstrated, the possibility seems more likely that this fat originates from intestinal secretion rather than from sloughed-off mucosa as postulated by Mead (3). As further evidence for this view, fecal fat that was fractionated after administration of acetate-C¹⁴ showed that approximately 50 percent of the total count was in the free fatty acids and monoglycerides, with the remaining 50 percent in the di- and triglycerides in both irradiated and control animals.

Thus, the indications are that lipid of similar composition is being formed by both types of rats varying only in the rate of formation. An attempt to gain further evidence to support this concept by the administration of acetate-1-C¹⁴ and the analyses of the material isolated from the lumen of the small intestine in normal and irradiated rats is being contemplated.

MARGARET G. MOREHOUSE

RONALD L. SEARCY

Department of Biochemistry and
Nutrition, University of Southern
California, Los Angeles

References and Notes

1. This research was supported by a grant from the U.S. Atomic Energy Commission, contract AT(11-1)-113, project 6, and conducted through the laboratory facilities of the Allan Hancock Foundation.
2. E. C. Dodds and J. H. D. Webster, *Lancet* 1, 533 (1924).
3. J. F. Mead *et al.*, *J. Nutrition* 43, 485 (1951).
4. J. G. Coniglio *et al.*, *Am. J. Physiol.* 172, 86 (1953).
5. G. T. Lewis and H. C. Partin, *J. Lab. Clin. Med.* 44, 91 (1954).
6. F. H. Mattson and L. W. Beck, *J. Biol. Chem.* 214, 115 (1955).

14 December 1955

Precipitation of Enzymes during Isolation of Chloroplasts in Carbowax

Two major difficulties in the determination of the intracellular distribution of enzymes are (i) the adsorption of soluble enzymes on particulate matter and (ii) the leaching of enzymes from the particles during the isolation procedures. It has been suggested that leach-