more than 80 percent of the new admissions of schizophrenics, the diagnosis was predictable from the chromatographic results.

Sano (*3*) has reported that the cold Millon's test, the Davis reaction, and the Mitsuba reaction are positive in some 40 to 50 percent of the urines of schizophrenics as opposed to 1 to 5 percent of the urines of normal individuals. Our evidence to date indicates that the results from these tests generally correlate well with the chromatographic ratings, but that the chromatographic ratings give a slightly more sensitive differentiation between schizophrenics and nonschizophrenics.

Reported R_f values for histidine derivatives in the solvent system used suggest that histidine derivatives are not responsible for any of the spots at $R_f > 0.1$. Confirming evidence has been obtained from studies of the changes in excretion pattern of persons on restricted diets (*6*). Four of the chromogens have been tentatively identified as indican, 3-hydroxyanthranilic acid, dihydroxyphenylalanine, and 5-hydroxyindoleacetic acid. Further studies are in progress .

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Studies of Chlorotetracycline Biosynthesis and the Preparation of Chlorotetracycline-C^{14}

As part of an investigation of the metabolism of *Streptomyces aureofaciens* Duggar, a number of studies have been made of the sources and intermediate states of the carbons in the chlorotetracycline (CTC) (*1*) that is accumulated by this organism. The organism studied was a mutant designated as *S. aureofaciens* BC-41, which is a descendant, through a series of mutation treatments, of the original A-377 soil isolate of Duggar.

Table 1. Carbon-14 incorporation into chlorotetracycline.

Labeled substrate	Incorporation (48-hour addition) (%)
Starch-C^{14}, uniformly labeled (*8*)	17.3
D-Glucose-1-C^{14} (*4*)	8.6
D-Glucose-2-C^{14} (*4*)	6.3
D-Glucose-6-C^{14} (*4*)	14.5
D-Glucose-C^{14}, uniformly labeled	12.2
D-Fructose-1,6-C^{14} (*4*)	3.4
Glycine-2-C^{14} (*8*)	52
L-Methionine-CH_3-C^{14}	48
D,L-Serine-3-C^{14}	18
Glycerol-1-C^{14}	6.5
Sodium acetate-1-C^{14}	13
Sodium acetate-2-C^{14}	15
Ethanol-2-C^{14}	7.4
Sodium formate-C^{14}	3.8
Formaldehyde-C^{14}	3.2

Measurements were made of the extent of carbon-14 transfer from a number of C^{14}-labeled metabolites to the carbon skeleton of CTC. For these experiments, the nutrient medium contained, as carbon sources, starch, corn-steep liquor, lard oil, and calcium carbonate (*2*). The weight ratio of labeled substrate added to CTC that was subsequently formed was less than 0.04. The CTC from each fermentation was isolated chromatographically. The product's radioactive purity was demonstrated by catalytic hydrogenation (*3*) of the radioactive CTC to a subsequently chromatographically isolated tetracycline (TC) of the same molar radioactivity.

Those metabolites significantly incorporated are presented in Table 1. Metabolites not significantly incorporated (< 3 percent) when added at zero hours were D,L-alanine-2-C^{14}, D,L-histidine-2-C^{14}, D,L-leucine-2-C^{14}, D,L-glutamic acid-2-C^{14}, D,L-methionine-2-C^{14}, adenine-8-C^{14}, guanine-8-C^{14}, urea-C^{14}, glycerol-1-C^{14}, sodium acetate-2-C^{14}, sodium carbonate-C^{14}, and phenol-1-C^{14}. Substrates not significantly incorporated when added at 48 hours, a time of rapid CTC production, were D-glucitol-1-C^{14} (*4*), L-arabinose-1-C^{14} (*4*), D-arabinose-1-C^{14} (*4*), D-arabinose-5-C^{14} (*4*), D-ribose-1-C^{14} (*4*), D-xylose-1-C^{14} (*4*), lactic acid-1-C^{14}, lactic acid-2-C^{14}, succinic acid-2-C^{14}, glycine-1-C^{14}, sodium carbonate-C^{14}, and shikimic acid-C^{14} (*5, 6*).

The CTC prepared from a fermentation to which starch-C^{14} had been added at the beginning of the fermentation cycle had a specific radioactivity 0.8 to 0.9 that of the starch, indicating that, under these fermentation conditions, 80 to 90 percent of the chlorotetracycline carbon originated from starch. The incorporation data can be considered evidence against a pathway from starch to CTC involving either fructose, pentoses, the Krebs cycle, carbon dioxide fixation, or shikimic acid (*6*).

The good incorporations observed from a number of metabolites known to be sources of one-carbon groups and the marked differences in extent of incorporation observed between the two differently labeled glycines and the two methionines indicate a role in the biosynthesis for one-carbon groups. The possibility of preferential appearance of carbon-14 from such donors in the 4-dimethylamino group of CTC was tested by degrading CTC, which was prepared from a fermentation containing glycine-2-C^{14}, with alkali (*7*) and isolating the resulting dimethylamine. Forty percent of the CTC radioactivity was found in the 4-dimethylamino group, which was approximately 4 times that expected from random labeling.

Pure, crystalline chlorotetracycline-C^{14} has been isolated in good yields from fermentations carried out in the presence of starch-C^{14} and in the presence of glycine-2-C^{14}. The specific radioactivity of the CTC that results is limited only by the radioactivity of the starch or glycine available.

The carbon-14 incorporations observed from the afore-mentioned simple substrates suggest that *S. aureofaciens* has the capacity to build the complete CTC molecule from simple materials. Related experiments have shown that *S. aureofaciens* spores can germinate, form mycelium, and biosynthesize CTC in a glycerol-mineral medium containing glycerol as the sole source of carbon and containing ammonium ion as the sole source of nitrogen. The concentrations of CTC accumulated during the glycerol-mineral-supported fermentation cycles were less than those accumulated during the corn-steep-supported cycles, but they show clearly that the organism is able to construct the entire CTC structure from simple beginnings.

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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-C^{14} added to the CTC subsequently formed was 0.55.
6. In the fermentations containing shikimic acid-C^{14} 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.
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8. When added at zero hours, starch-C^{14} (uniformly labeled), D,L-lysine-2-C^{14}, D,L-ornithine-2-C^{14}, and glycine-2-C^{14} showed 10, 8.7, 4.2, and 12 percent incorporation, respectively.

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Shell Mineralogy in Paleozoic Invertebrates

Among the modern invertebrate groups that deposit shells of $CaCO_3$, 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 calcitic 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 bellerophontaceid 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 bellerophontaceid 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 *Trepospira*.

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-