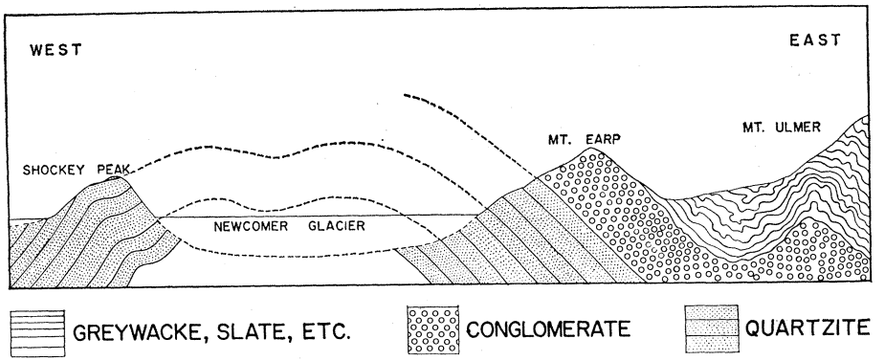

Fig. 2. Generalized west-east geologic cross section from Shockey Peak to Mount Ulmer, northern Sentinel Range.

sections studied, and possible fossils, mainly fucoidal impressions and probable worm borings, were discovered at only 12 localities. Most of these specimens are poorly preserved, and no generic or specific identifications have been made as yet. This material is receiving further study, and the age of these sedimentary rocks is at present unknown.

The strata of the Sentinel Range have been deformed into a complex set of folds whose axes plunge about 5° to N.10°–20°W., roughly parallel to the trend of the mountains (Fig. 2). Most of these folds are asymmetric or overturned; the axial planes generally dip steeply to the east although recumbent folding is also present. The large folds defined by the principal statigraphic units have wavelengths of up to 2 miles. Superimposed upon these major folds are many smaller ones, especially in the lower and upper of the three units, with wavelengths of tens or hundreds of feet; these smaller folds are commonly tight and locally isoclinical. Important faulting has not been demonstrated in these strata, although one steep strike fault of indeterminate displacement was observed in a spur along the western base of the Sentinels. All the original sedimentary rocks have undergone low-grade metamorphism, and locally they have developed secondary, steeply inclined foliation.

The northern Heritage Range consists mainly of quartzites believed to correlate with the lowest unit in the Sentinels. A typical rock contains mainly quartz grains with minor sodic plagioclase, sericite, and chlorite and traces of titanium oxide, iron oxide, apatite, epidote, zircon, calcite, muscovite, and microcline. Layers (1 mm) of detrital heavy minerals, mainly rounded garnet and iron oxide, occur in the quartzites. White quartz veins with epidote and chlorite are abundant. In the northeastern part of the range five basic sills up to 100 feet thick have intruded these strata.

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10 September 1962

Method of Introducing Tritiated Thymidine into the Tissues of Hydra

Abstract. Although normal hydras resist the uptake of tritiated thymidine into their tissues, regenerating forms contain a very heavy label in interstitial cells, epitheliomuscular cells, and digestive cells of the gastrodermis if the animals are allowed to regenerate in a medium containing the isotope. Preliminary results suggest that during early regeneration active wound repair is not effected by interstitial cells which divide and then begin to differentiate, but through differentiation of interstitial cells which are nondividing or which have partially begun to differentiate. Dividing interstitial cells probably function to restore the animal's original embryonic reserve.

The hydra is virtually impermeable to most exogenous free compounds. This impermeability is probably due to the mucous coat which covers the entire epidermis of the animal. Traditional vital dyes, such as methylene blue, penetrate the tissues of the animal extremely slowly unless the animal is immersed in highly concentrated solutions. To our knowledge, the only radioactive materials which have been found to penetrate the tissues of the animal from the external medium are labeled glycine (*1*) and carbon dioxide (*2*), both of which are small molecules.

Our attempts to introduce tritiated thymidine into intact animals had been singularly unsuccessful. However, we have recently devised a simple method for introducing this isotope which we feel will be applicable to other coelenterates and to flatworms, which, because of their mucous coat, are also highly impermeable.

The species of hydra employed in the following experiment was *Hydra pseudoligactis*. The animals were reared by the method of Loomis and Lenhoff (*3*). Animals chosen for experimental purposes were starved 24 hours, and then excised through the middle of the gastric region or just beneath the hypostome. The proximal portions of the hydras were then placed in 50 ml of Versene-water buffered with sodium bicarbonate (*3*). Tritiated thymidine, in a concentration of 0.5 mc/ml, was added to the solution to produce a specific activity of 10 μc/ml. Regenerating animals were left in the incubating medium for periods of 10 minutes, 30 minutes, 1 hour, 2 hours, 6 hours, 9 hours, 12 hours, 18 hours, 24 hours, and 48 hours. After removal from the solution the hydras were washed thoroughly in three changes of Versene-water, relaxed in 10-percent alcohol, and fixed in Bouin. The animals were then dehydrated, embedded in paraffin, and sectioned at 5 μ. The sections were then coated with Kodak type NTB3 No. LSB3-9-1 emulsion, dried, and placed in a deepfreeze for 2 weeks. The emulsion was developed in Dektol, fixed in hypo, washed, stained in 0.5-percent toluidine blue buffered to *p*H 8, destained in 50- or 70-percent alcohol, cleared in xylene, and mounted in Permount (*4*).

Animals incubated in the thymidine for 10 minutes showed a very significant label in interstitial cells and digestive cells of the gastrodermis. No cell was counted as labeled unless there

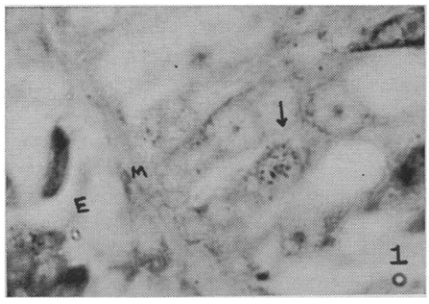

Fig. 1. Uptake of tritiated thymidine into nucleus of a gastrodermal digestive cell (arrow). Toluidine blue, *p*H 8, about × 600; *M*, mesogloea; *E*, epidermis.

were at least ten grains located directly over the nucleus. A few epitheliomuscular cells of the epidermis were also labeled during this 10-minute period, but usually no more than two or three labeled cells of this type were found in one section in contrast to interstitial cells and digestive cells which contained from ten to 25 labeled members per section. All of the cell types labeled during this period were found immediately adjacent to the wound area.

Animals exposed for longer periods, 6 to 12 hours, were labeled profusely not only in the wound area but along the length of the gastric region as well. In each case the label was limited to interstitial cells, epitheliomuscular cells of the epidermis, and digestive cells of the gastrodermis. On a few occasions, cells resembling mucous cells of the gastrodermis appeared to be labeled, but more investigation will be necessary to confirm this observation. At no time was label sighted in the peduncular region of the animal or in the basal disk, but it is possible that the isotope had not diffused to this area at the time of observation.

At the 24-hour stage label was sighted in cnidoblast cells, mucous cells, and gland cells of the gastrodermis. This observation suggested that these cell types, since they did not take up the label during earlier stages, were perhaps formed by the differentiation of interstitial cells. This observation was strengthened by the fact that animals which remained in the thymidine for 1 hour and were then removed and washed thoroughly and then fixed at various intervals did not contain label in the cnidoblast or gland cells until the 18- to 24-hour period.

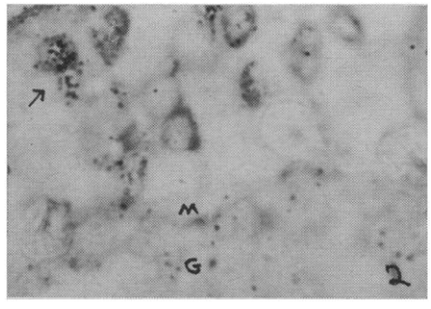

Fig. 2. Uptake of tritiated thymidine into two interstitial cells. Toluidine blue, *p*H 8, about × 600; *M*, mesogloea; *G*, gastrodermis.

The foregoing observations confirm the fact that interstitial cells are not the sole autoreproductive elements of hydras (*5*, *6*), and casts some doubt upon the concept of "totipotency" of the interstitial cell. It is quite conceivable that interstitial cells are capable of differentiating into only a few select cell types, and during the life of the hydra never differentiate into epitheliomuscular cells and digestive cells.

One observation made during the course of these studies deserves special mention. It was of common occurrence throughout all of the stages examined to find that entire nests of interstitial cells were labeled. These nests may be composed of 12 to 15 cells. Moreover, it was observed that during the early stages of regeneration most of the interstitial cells which had begun to differentiate, that is, had grown three or four times in size and taken on a spindle shape, were unlabeled. Burnett (*6*) has reported that animals whose interstitial cells have been selectively destroyed are still able to regenerate if they contain a large reservoir of partially differentiated interstitial cells in their tissues. The lack of label in interstitial cells which have already begun to differentiate suggests strongly that these cells did not arise from interstitial cells which divided in response to the wound stimulus. It is quite possible that the dividing interstitial cells function mainly to restore the original embryonic reserve of the animal and do not contribute directly to the early processes involved in tissue repair.

Now that tritiated thymidine can easily be introduced into the tissues of hydras, it should be possible to trace the entire sequence of cell differentiation in this form. This may be accomplished by labeling the interstitial cells of a regenerating animal and grafting this animal to a hydra whose interstitial cells have been selectively destroyed by nitrogen mustard or irradiation with x-rays. Interstitial cells readily migrate into the host animal and repair the irradiation damage. An examination of the host animals over different time intervals will reveal the differentiated cell types which contain the label. Experiments of this type are under way in our laboratory at the present time.

In conclusion, although it is not known with certainty why hydras "leak" during the regeneration period, it appears that a simple excision through the body column may be an extremely effective method of introducing relatively large molecules into the tissues of the animal. Initial attempts to introduce tritiated uridine by this method have also been successful (*7*).

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Archeological Investigations in East Central Arizona

Abstract. A blend of traditional and new methodologies is being employed to analyze extinct cultural systems up to 4000 years old. A Univac computer was used to demonstrate a functional attribute of ceramics, and sociological inferences were made from spatial distributions of pottery designs.

For six years a group of us at the Chicago Natural History Museum (*1*) have been making a broad-scale study of the prehistory of eastern Arizona—specifically in the area lying between the towns of Snowflake, Show Low, Springerville, and St. Johns. Our basic objectives have been to understand and to explain similarities and differences in patterns of culture and the processes by which these patterns have developed from simple to more complex. We hope to correlate the adaptation of the extinct cultural systems under investiga-