Wales, as well as the Antarctic, indicates a general condition of limited water bodies of short duration in the Gondwana area, with comparable weather (as distinct from climatic) cycles in these areas. Vertically restricted occurrence of leaiids in a single zone is known in South America (Rio do Rasto Formation) and South Africa (Lower Beaufort), as well as Antarctica (Mt. Glossopteris Formation). Their wide range in the Newcastle Coal Measures of New South Wales may be attributed to the recurrence of temporary water bodies. In turn that would suggest a longer enduring climatic effect in New South Wales than elsewhere in Gondwana.

The Antarctic *Leaia* zone contains conchostracans with discernible fossil eggs, and some unidentifiable fossils which appear to be ostracods. *Leaia* n. sp. and *Cyzicus* (*Lioestheria*) n. sp. (*3*) are the two main conchostracan components. *Leaia* n. sp. bears many similarities to *L. pruvosti* Reed of Brazil (*4*), and strong resemblances to *L. compta* Mitchell of New South Wales (*5*). *Cyzicus* (*Lioestheria*) n. sp. may be compared with *C.* (*Lioestheria*) *belmontensis* (Mitchell), though it differs from the latter in configuration.

When one considers Gondwana deposits in general, the occurrence of leaiid conchostracans in the Lower Beaufort (Middle-Upper Permian) of South Africa, South America, and Australia appears to be a rather distinguishing feature; and the apparent absence of *Leaia* species above this horizon also assumes significance when one attempts to pinpoint the age of the Antarctic leaiids. Although not infrequent in the Carboniferous outside the Gondwana area, conchostracans assignable to the genus *Leaia* have never been reported from the Gondwana Carboniferous; and the members of this genus have never been reported in beds younger than Permian.

In South Africa (*6*), South America, Australia, and Antarctica, the similarities among the leaiid species, the association of leaiids with *Glossopteris* components, and the many identical species of the *Glossopteris* flora, establish a Lower Beaufort age for the leaiid zone of the Ohio Range (*7*).

GEORGE A. DOUMANI*
*Institute of Polar Studies,*
*Ohio State University*

PAUL TASCH
*Department of Geology,*
*University of Wichita, Wichita, Kansas*

**References and Notes**

1. W. E. Long, *Science* **135**, 319 (1962).
2. J. M. Schopf, *Ohio State Univ. Inst. Polar Studies No. 2* (1962); A. A. Cridland, *Am. J. Botan.* **50**, 186 (1963).
3. *Lioestheria* has been reclassified as a subgenus of *Cyzicus*: P. Tasch, in *Treatise on Invertebrate Paleontology*, in press. The Antarctic leaiids are described and figured in a detailed report by G. A. Doumani and P. Tasch which is in preparation.
4. F. R. C. Reed, *Serv. Geol. Min. Brasil Bol. No. 34* (1929); J. S. Mendes, *Museu Paranaense*, Brazil (1954).
5. J. Mitchell, *Proc. Linnean Soc. N. S. Wales* **1**, 438 (1925) vol 1.
6. K. H. Barnard, *Ann. S. African Museum* **22**, 181 (1931).
7. Contribution No. 37 from the Institute of Polar Studies. The work was supported by National Science Foundation grants G-13590 and G-17216. Logistical support provided by U. S. Naval Task Force 43.

* Present address: Science and Technology Division, Library of Congress, Washington 25, D.C.

22 July 1963

## Glycogen-Containing Cells of Estrogen-Induced Renal Tumors of the Hamster

Abstract. *Cells which contain glycogen, and which may arise from the connective tissue matrix, can be demonstrated in the estrogen-induced renal tumor of the hamster when the tumor tissue used has either been lyophilized or frozen and then substituted in absolute alcohol containing mercuric chloride. Glycogen has not been found previously in this type of tumor. Studies of the role of glycogen in this type of neoplasm may elucidate mechanisms of tumorigenesis.*

Although glycogen has been found in the cells of some renal tumors in humans (*1*), Kirkman, as well as Horning and Whittick (*2*), were unable to demonstrate glycogen in the estrogen-induced renal tumor of the hamster. They used a large number of different fixatives and specific stains for glycogen, including Best's carmine and the periodic acid-Schiff reaction (PAS). The study presented here shows that in estrogen-induced renal tumors in our hamsters, there are cells containing glycogen which can be demonstrated when frozen-dried or freeze substituted tumor tissues are stained with the periodic acid-Schiff reagents.

A hamster carrying the 80th transfer of an estradiol-induced and estrogen-dependent renal tumor was obtained from Kirkman (*3*) and the tumor was transplanted subpannicularly into male hamsters treated with stilbestrol. Subsequent transplantation of the Kirkman tumor has resulted in two strains of tumor, one of which is autonomous, the other being the stilbestrol-dependent strain. The original Kirkman tumor, as well as the transplanted dependent and autonomous tumors, were removed from the hosts and prepared for study by freezing in isopentane cooled with liquid nitrogen to −155°C. Tissue was then either dried in a vacuum according to a technique described previously (*4*) or substituted in absolute alcohol containing 1 percent mercuric chloride kept at −70°C for about 7 days. Paraffin-embedded tissue was then sectioned and mounted on albumenized slides without contact with aqueous solutions. Petroleum ether was used to remove the paraffin from the sections, which were afterward placed in absolute alcohol overnight. A section was then placed in a 0.1-percent diastase solution in buffer, p*H* 7.0, for 1 to 2 hours at 37°C. An adjacent section similarly

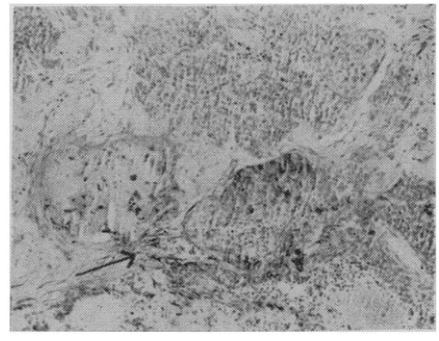

Fig. 1. Section through renal tumor of hamster, showing glycogen-containing cells as black granules. The arrow indicates septal cells. (× 75)

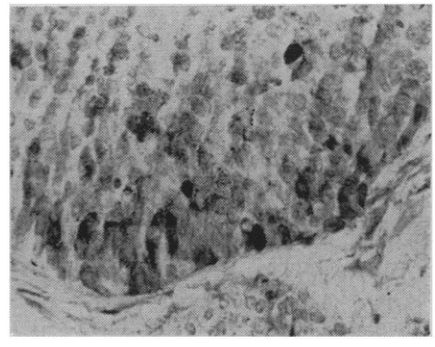

Fig. 2. Section showing glycogen in mass of tumor cells. (× 300)

prepared in petroleum ether and absolute alcohol was placed in a solution containing only buffer at *p*H 7.0 for the same time. These sections were then again placed in absolute alcohol overnight and afterward stained with the periodic acid-Schiff reaction. Another section was placed in absolute alcohol overnight and stained with PAS to show the total PAS-reactive material. Sections were counter-stained with malachite green. Sections from the original Kirkman tumor and from twenty transplants performed in our laboratory were examined.

Dark purplish-red granules, which are black in the accompanying photomicrographs, may be seen in the large cells which are scattered sparsely throughout the tissue section (Fig. 1). Clusters of these cells are present not only in the connective tissue septa between tumor cell masses (see arrow, Fig. 1), but also in the tumor mass (Fig. 2). The PAS-positive granules in these cells were removed by the diastase digestion but were not removed by the buffer alone. Most of the tumor cells in this study were not PAS-positive although Kirkman (*2*) found the majority of hamster renal tumor cells to be PAS-positive. The glycogen cells described here were found in the autonomous and the estrogen-dependent tumors and were seen in all the sections examined.

These cytochemical studies thus show that glycogen is present in some of the cells of estrogen-induced renal tumors in the hamster. Others have not found glycogen in these tumors, but the methods of fixation that they used may have removed this relatively soluble carbohydrate from the tissue section. Fortner (*5*) described clusters of large polygonal cells in a bile-induced (*6*) renal tumor in the hamster. These cells contained large amounts of clear or granular cytoplasm and could, indeed, represent the glycogen-containing cells described in this paper. The question naturally arises as to whether these glycogen-containing cells are altered tumor cells, or specialized cells from the connective tissue matrix. The preponderance of these cells in the connective tissue septa suggests that they may arise from this connective tissue matrix.

Renal tumors in hamsters and in humans are morphologically similar. Since they are histochemically similar as evidenced by the presence of glycogen-containing cells in both, the question arises as to whether the mechanisms of tumor induction are also similar. Thus, mechanisms of tumorigenesis may be elucidated by a study of the intermediate pathways of glycogen metabolism of these tumors (*7*).

JOHN A. ARCADI
*Department of Biology, Whittier College, Whittier, California*

**References and Notes**

1. O. Lubarsch, *Arch. Pathol. Anat. Physiol.* **183**, 188 (1906).
2. H. Kirkman, *Natl. Cancer Inst. Monograph No. 1* (1959); E. S. Horning and J. W. Whittick, *Brit. J. Cancer* **8**, 451 (1954).
3. I thank Dr. Hadley Kirkman for generously supplying the original tumor, and for counsel.
4. J. A. Arcadi and C. Tesar, *J. Lab. Clin. Med.* **43**, 479 (1954).
5. J. G. Fortner, A. G. Mahy, R. S. Cotran, *Cancer Res.* **21**, 199 (1961).
6. This tumor may have been induced by the estrogen contained in the bile.
7. Aided, in part, by a grant from the Southern California Chapter of the National Kidney Disease Foundation, by grant C-4054 from the National Cancer Institute and from the Ballantyne Grant of the Whittier College Biological Research Fund. This paper was presented to the Johns Hopkins Medical and Surgical Society, Brady Residents meeting, 2 March 1963.

17 July 1963

## Chlorinated Insecticides in the Body Fat of People in the United States

Abstract. *Benzene hexachloride and dieldrin are present in the body fat of people in the general population of the United States. The mean concentration of dieldrin is 0.15 ± 0.02 parts per million, which is in good agreement with the concentration reported for southern England. The mean concentration of benzene hexachloride is 0.20 ± 0.04 ppm, which is considerably lower than the mean concentration reported for France. Paired analyses of DDT by gas chromatographic and colorimetric methods show that the results of the latter may give incorrectly high results when applied to human fat.*

Both DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl) ethane] and its metabolite DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene] are present in the body fat of people in the general population of the United States (*1, 2*) and other countries (*3–5*). It was shown recently, by the use of gas chromatography and highly sensitive detectors, that dieldrin (*4*) (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) and benzene hexachloride (*5*) (1,2,3,4,5,6-hexachlorocyclohexane), respectively, are present in the body fat of people in southern England and France. No information has been available as to the occurrence of chlorinated pesticides, other than DDT and DDE, in the body fat of the general population of this country, but, because the average concentrations reported for DDT and DDE in the United States (*2*) were higher than those reported for other countries (*3–5*), we suspected that other chlorinated pesticides might also be present. Therefore, in connection with a periodic survey carried out in 1961–62 to determine whether there has been any change in the storage of DDT in people of this country, those samples of fat that were large enough were analyzed not only by the Schechter-Haller method (*6*) but also by microcoulometric gas chromatography (*7*) to detect other insecticides.

The results obtained by the Schechter-Haller method (*6*) for DDT and DDE in 131 samples from the general population and 51 other samples from people with occupational or other special exposure are to be reported separately (*8*). The two autopsy and 28 surgical samples reported here (Table 1) were collected in Wenatchee, Washington; Phoenix, Arizona; and Louisville, Kentucky. All but four of the subjects from whom the samples were obtained had been residents of the respective states at least 2 years; only two (samples 29 and 30) had experienced occupational exposure to pesticides. In no instance was illness related to pesticides.

For this study, samples of body fat (18 to 42 g) were taken for comparative study by Schechter-Haller colorimetric analysis, and by gas and paper chromatography. These samples were chopped and extracted in a Waring blender with *n*-hexane. After extraction was complete, sodium sulfate was added and mixing was continued for 5 minutes to remove moisture. The extracts were then filtered and given a preliminary cleaning by partitioning with acetonitrile (*9*).

Gas chromatograms (Fig. 1, *A* and *D*) of standard solutions showed that dieldrin and DDE have the same retention times on the columns used. Since we were interested in determining both the dieldrin and DDE in fat by gas chromatography, a preliminary separation of these compounds was required.

A column in which florisil is used has been described by McKinley *et al.*