mμ. This differential rate of production of $NADPH_2$ was taken as the measure of glucokinase activity and expressed as micromoles of $NADPH_2$ per gram (wet weight) of liver per minute (extinction coefficient of reduced NADP of $6.22 \times 10^6$ $cm^{-2}$ $mole^{-1}$). The glucose concentration used in these experiments (25m*M*) was well above that used by Vinuela *et al.* (*3*) for hexokinase, though it was still not optimal for glucokinase. This glucose concentration was selected arbitrarily for these studies. In addition, the rate of phosphorylation (Fig. 1) of 1.51 ± 0.15 μmole of $NADPH_2$ per gram of liver per minute, when corrected by the method described by these authors (3), is such that approximately 1.0 μmole of glucose per gram of liver per minute will be phosphorylated at this substrate concentration. This is more than double the highest rate of phosphorylation of glucose by hexokinase observed (*3*) (0.46 μmole per gram of liver per minute) and therefore the rate must depend on glucokinase to a large extent.

The effect of various concentrations of insulin on the glucokinase activity of rat liver supernatants is shown in Fig. 1. The data represent the differences between the values obtained in the presence and absence of insulin. It is apparent that the response to insulin is dependent upon the dose within the given limits.

In order to determine whether or not the response to insulin was due to some nonspecific protein effect analogous to that observed with yeast hexokinase (*7*), the effect of bovine serum albumin was studied. In five trials, no significant effect was noted with concentrations equivalent to 300 milliunits of insulin per milliliter or less. Likewise, in 20 trials, glucokinase activity was not stimulated by insulin inactivated with heat and alkali. With concentrations equivalent to 300 milliunits of insulin per milliliter, inactivated insulin produced a slight inhibition of glucokinase. That the insulin effect results from specific action on glucokinase is supported by the lack of response (preliminary studies) to insulin of liver supernatants prepared from the livers of three diabetic animals which appeared to possess only hexokinase activity (*3*).

The response of the extracts to insulin was very labile. Thus, any deviations in the control of temperature diminished the responsiveness to insulin. For full effects, the time between killing the animal and beginning the incubation studies must be kept to 2.5 hours or less. Incubation of the supernatant at 37°C for 30 minutes prior to the addition of insulin abolished the response completely. Similar insulin effects at both higher (0.1*M*) and lower (0.008*M*) concentrations of glucose have been noted (*8*).

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## Leaiid Conchostracan Zone in Antarctica and Its Gondwana Equivalents

Abstract. *Two species of conchostracans (class Crustacea) occur in the coal measures of the Ohio Range, associated with a typical* Glossopteris *flora. New* Leaia *and* Cyzicus (Lioestheria) *species occur in a restricted zone near the top of the stratigraphic section. The biofacies represents a swampy environment and deposition in short-lived ponds of still water under variable weather cycles. The zone compares closely with the leaiid zones in South Africa, South America, and Australia, and is assigned to the Middle-Upper Permian, specifically, Lower Beaufort age.*

The stratigraphic section at the Ohio Range, Antarctica, was described previously, and formation names were assigned to the different lithologic units (1). The section is capped with a diabase sill which overlies the Mount Glossopteris Formation at Mercer Ridge (Fig. 1). About 160 m below the sill is a 1.5-m bed of black, carbonaceous shale, well indurated but intensely and unevenly fractured, with surfaces weathered to white. The conchostracans were collected from a vertically-restricted zone, 10-15 cm thick, which extended laterally for about 60 m. The shale is intercalated with cross-bedded, arkosic sandstones, carbonaceous shales, thin coal seams, and lenticular bodies of quartz-pebble conglomerate.

The spotty lateral distribution of conchostracan fossils suggests a habitat in which there were bodies of water of the size of puddles and ponds. The fossil assemblage includes a typical *Glossopteris* flora (2), carbonized wood, as well as some carbonized leaiid valves, all of which suggest swamp conditions. The flat-lying, uncarbonized leaves that are associated with uncarbonized conchostracan valves indicate deposition in shallow, still water.

Seasonal events are deduced from the study of sediment-intervals between six successive conchostracan generations. The study reveals a sedimentation rate of 0.68 mm per year for the Antarctic *Leaia* zone, and an intermittent occupancy of water bodies during Lower Beaufort time. The length of a season, as determined from growth bands, shows that leaiids apparently thrived in the existing pools of the time from 21 to 48 days, and lioestheriids from 27 to 30 days. The 1 to 1½-month duration suggests that the ponds or pools also were short-lived. The size range of leaiids from beds of equivalent age in Brazil and New South

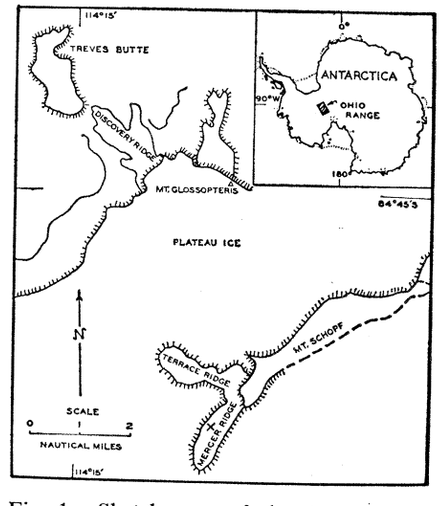


Fig. 1. Sketch map of the Ohio Range, Antarctica. The locality where fossil conchostracans and associated *Glossopteris* components were collected from the west face of Mercer Ridge is indicated by ×.

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*).

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## 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

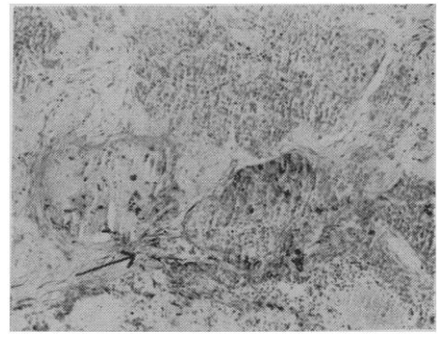

Fig. 1. Section through renal tumor of hamster, showing glycogen-containing cells as black granules. The arrow indicates septal cells. (× 75)

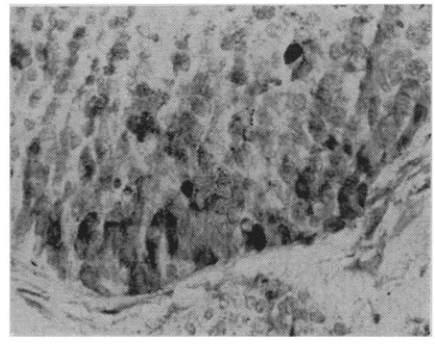

Fig. 2. Section showing glycogen in mass of tumor cells. (× 300)