did not react with the CRS implanted in the skin.

The intravenous injection of 10 ml of the reaginic serum passively sensitized normal dogs for anaphylaxis resulting from subsequent injection of 1 ml of a 3 percent extract of ragweed pollen. Twenty ml of the IDS, given simultaneously with sensitizing reaginic serum or after incubation with the challenge dose of ragweed antigen, showed that the normal dogs were protected from anaphylaxis. Table 1 summarizes the results of the sensitization and protection experiments.

Passive cutaneous reactions were eliminated by mixing IDS with the challenging ragweed antigen. This alteration in the cutaneous reaction occurred when the control reaction either preceded or followed the protection experiment. Thus, neutralization of reagin by persisting antigen after a control experiment was not responsible for the inhibition. A large increase in the quantity of ragweed antigen used for challenge resulted in reversal of inhibition of the cutaneous reaction by IDS. Dogs were not resensitized for anaphylaxis until a positive passive cutaneous reaction could be elicited.

Table 1. The effect of IDS (serum from dogs immunized against ragweed) in animals passively sensitized with CRS (canine reaginic serum) obtained from dogs spontaneously sensitive to ragweed pollen. The challenge dose of ragweed was 1 ml of 3 percent solution. The amount of IDS was 20 ml, and the amount of CRS was 10 ml.

Dog No.	Sensitizing material	Intravenous challenge after 24 hrs	Severity of symptoms after challenge*
1	CRS-1	RW	3+
	3-Day interval		
	CRS-1 + IDS	RW	None
	14-Day interval		
	CRS-1	RW	3+
	9-Day interval		
	CRS-1	RW + IDS	None
2	CRS-1	RW + IDS	±
3	CRS-1 + IDS	RW	None
4	CRS-1	RW + normal dog serum	3+
5	CRS-1 + 20 ml normal dog serum	RW	3+
	9-Day interval		
	CRS-1 + IDS	RW	None
6	CRS-2	RW	2+
	3-Day interval		
	CRS-2 + IDS	RW	None
7	CRS-2	RW + IDS	None
8	CRS-2	RW	2+

* 1+ = Ataxia, vomiting or diarrhea or both; 2+ = Ataxia, vomiting, diarrhea, and collapse; 3+ = Ataxia, vomiting, bloody diarrhea, collapse, dyspnea.

Thus, antibodies can be induced in normal dogs injected with pollen antigen which will protect dogs passively sensitized against ragweed antigen by canine reagin.

JAMES I. TENNENBAUM
ROY PATTERSON
JACOB J. PRUZANSKY
Northwestern University Medical School, Chicago 11, Illinois

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9. Supported by grant AI-01358 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

8 July 1963

Hepatic Glucokinase: A Direct Effect of Insulin

Abstract. *Particle-free extracts of the livers from rats on a normal diet were incubated with insulin for 4 minutes; during this time the glucokinase activity increased. This insulin effect is dependent upon the dose.*

The utilization of glucose by the liver of the alloxan-diabetic rat is profoundly impaired (*1*). This impairment is not due to a blocking of the transport of glucose into the hepatic cell (*2*). Since the glucokinase activity of the liver of the alloxan-diabetic rat is diminished (*3*), it is possible that this decrease in enzyme activity is responsible for the impairment in glucose utilization. In accord are our data, which show that, under specific conditions, insulin exerts an effect on hepatic glucokinase in vitro.

Male Holtzman rats weighing 150 to 250 g, maintained on a rat chow diet, were used. After the animals had been stunned and decapitated, the liver was perfused *in situ* with 40 ml of cold (4°C) 0.15*N* NaCl solution.. All procedures were conducted at 4°C. The liver was removed and blotted on filter paper, weighed, minced with scissors, and homogenized in 0.3*M* KCl in a Potter-Elvehjem Teflon-glass homogenizer. The homogenate was adjusted to a final volume of 4.0 ml per gram (wet weight) of liver. It was centrifuged for 10 minutes at 20,000*g* in a Servall SS-4 centrifuge to remove larger particles, and the supernatant was recentrifuged at 100,000*g* for 30 minutes in a Spinco model L preparative centrifuge.

Each supernatant was tested as follows: 0.05 ml of supernatant was added to each of four cuvettes. To two of these, water was added in a volume equal to that of the insulin solution used in the other pair—9 to 30 μl. To the other pair of cuvettes, insulin (25 units per milliliter) was added (*4*). Each pair of cuvettes was placed in a Beckman D-K double-beam recording spectrophotometer and incubated for 4 minutes. Solutions incubated in this fashion reach a temperature of 29° to 30°C within 1 minute and remain constant thereafter. At the end of the initial 4-minute interval, the glucokinase activity was determined by a modification of methods described elsewhere (*5*). Accordingly, the following reagents were added: 0.15*N* KCl in amount to make final volume 3.0 ml; 0.1*M* tris buffer (*p*H 7.6), 1.0 ml; $MgCl_2$, 16 μmole, 20 μl; hexokinase-free glucose-6-phosphate dehydrogenase from yeast, 0.3 Kornberg units (*6*), 10 μl; nicotinamide adenine dinucleotide phosphate (NADP), 2 μmole (*p*H 6.5), 20 μl; glucose, 75 μmole, 25μl.

The contents of the cuvettes were stirred after each addition, and the reaction was initiated in one cuvette by the addition of 10 μmole of adenosine triphosphate (the disodium salt) which had been adjusted previously to *p*H 6.5. The absorption difference between the two cuvettes was measured at 340

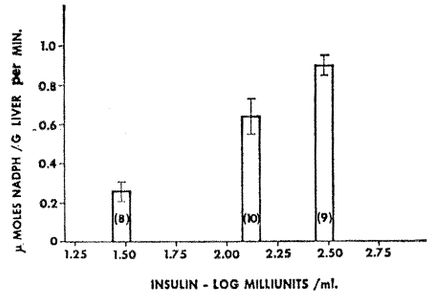

Fig. 1. The vertical bars indicate the mean (± S.E.) differences in glucokinase activity between untreated controls and supernatants treated with 30, 133, and 300 milliunits of insulin per milliliter. The numeral in each bar is the number of animals at that dosage. The mean rate of $NADPH_2$ production by the untreated controls (27) was 1.51 ± 0.15 μmole per gram of liver per minute. The ordinate is the increase in glucokinase activity attributable to insulin.

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 6.22×10^6 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*).

JOHN W. VESTER
MARY L. REINO
Departments of Medicine and
Biochemistry, University of
Pittsburgh School of Medicine,
and *Veterans Administration Hospital,*
Pittsburgh 40, Pennsylvania

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8. Supported in part by U.S. Public Health Service grant No. A-1708(C4).

6 August 1963

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

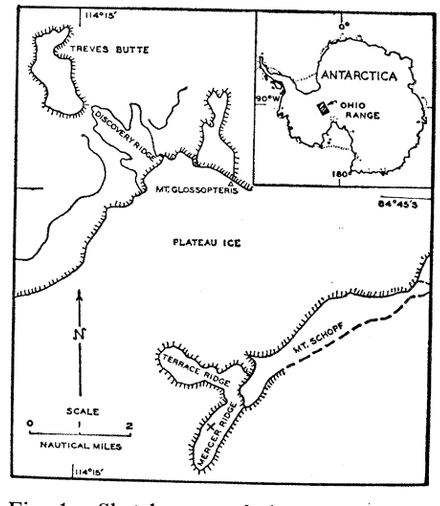

Fig. 1. Sketch map of the Ohio Range, Antarctica. The locality where fossil conchostracans and associated *Glossopteris* components were collected from the west face of Mercer Ridge is indicated by ×.