sufficiently large to account for the balance of the size-frequency array for fossil grains at Sodon Lake.

To Fig. 1 has been added a very useful type of graphic presentation of statistical data originated by Dice and Leraas (*4*). The data forming the basis for the diagrams are presented in Table 1. The horizontal line marks the range for each population. The transverse or vertical line marks the position of the mean ($\overline{X}$). The open rectangle marks off one standard deviation (σ) on each side of the mean; and the black, superimposed rectangle marks off 2 standard errors ($2\frac{\sigma}{\sqrt{N}}$) each side of the mean. It is thus apparent that there is no significant

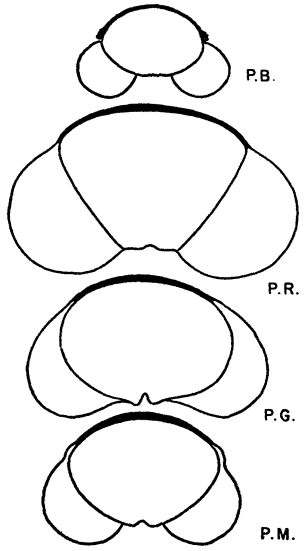

FIG. 3. Median optical views of pollen grains of the $\overline{X}$ size class for each species. (P. B.) *Pinus Banksiana* (45 μ), added for comparison with the spruces, (P. R.) *Picea rubra* (83 μ), (P. G.) *P. glauca* (77 μ), (P. M.) *P. mariana* (67 μ).

statistical difference between the two samples of pollen of the white spruce. It is equally obvious that the three species (in so far as they are represented by the presently available material) have pollen grains of such size that adequate samples easily permit their statistical separation, despite the considerable overlap of ranges.

Despite frequent confusion in the identification of the eastern American spruces, and the fact that the red spruce has sometimes been attributed to the western Great Lakes area in its modern distribution, there seems to be no doubt that today the species is nearly strictly Appalachian and New England in occurrence (Fig. 2), based on Munns (*6*). We face the question, then, whether the present data are sufficient to warrant the conclusion that the red spruce was present far west of its modern area during pre-Boreal time; specifically whether it was in the neighborhood of Sodon Lake, in southeastern Michigan. The hypothesis that it was is a reasonable one. It is also apparent, however, (*1, 2*) that different collections of pollen of the same species may have statistically significant size differences; hence, there is an urgent need for further size-frequency studies of these species. Verification of the size-frequency characteristics of these three species of *Picea* and further measurements of fossil grains in other profiles should strengthen the circumstantial case for the former extension of range of the red spruce.

Further evidence for the presence of fossil red spruce pollen grains in the Sodon Lake sediments lies in the forms of the grains, many of which match perfectly the typical form of this species (Fig. 3, P.R.). Pollen grains are somewhat variable in form, but the illustrations show scale drawings of grains that are typical both as to size and shape. It is not claimed that individual pollen grains of spruce can be identified and that percentage composition of the species can be determined for use in pollen spectra, but it does seem that by a combination of size and form characteristics, when numerous grains are available from a particular sediment, the presence of certain species can be detected.

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Inhibition of Glycolysis *in Vitro* by Impure Penicillin[1]

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It has been reported by Miller, Hawk, and Boor (*2*) that impure penicillin protects mice against bacterial endotoxins. Since it was found that bacterial endotoxins cause marked disturbances in the intermediate metabolism (*1*), it seemed of interest to search for metabolic effects of impure penicillin in the hope of gaining an insight into the mechanism responsible for its protective action.

Mouse liver and muscle and mouse sarcoma 37 were homogenized in ice-cold distilled water by means of glass homogenizers (*3*), and glycolysis was determined manometrically by the system of Utter, Wood, and Reiner (*4*). Each Warburg flask contained 20 mg of tissue homogenate. Glycolysis was expressed in terms of mm^3 of CO_2 formed in 20 min, after the homogenate was mixed with the contents of the main compartment. Impure penicillin[2]

[1] This investigation was supported by the U. S. Navy, Office of Naval Research, and the University of Chicago.

[2] An intermediate product in the commercial production of penicillin, kindly supplied by the Abbott Laboratories.

containing approximately 5,000 units of penicillin/ml (4.1% content of solids including phosphate buffer) was added in 0.5-ml volume/flask.

TABLE 1

INHIBITION OF GLYCOLYSIS OF MOUSE TISSUES BY IMPURE PENICILLIN

No. of experiment	Liver				Muscle				Sarcoma			
	Aerobic		Anaerobic		Aerobic		Anaerobic		Aerobic		Anaerobic	
	C*	IP*	C	IP	C	IP	C	IP	C	IP	C	IP
1	58	30	64	37	68	37	75	39	59	41	29	16
2	61	32	69	39	71	40	88	46	60	45	29	16
3	57	29	64	31	66	30	79	40	56	43	26	14
4	59	31	66	35	65	33	86	44	57	46	29	14
5	63	30	70	40	69	41	82	45				
Average	60	30	66.7	36	68	36	82	43	58	44	28	15
Difference	50%		46%		47%		48%		24%		47%	

* C = control; IP = impure penicillin added.

Results are given in Table 1. These show that both aerobic and anaerobic glycolysis of liver and muscle homogenates was equally inhibited, suggesting that the site of action of the impure penicillin is in the anaerobic part of the glycolytic cycle. The sarcoma gave a higher aerobic than anerobic glycolysis, which is typical for malignant tissues. The impure penicillin caused less inhibition of the aerobic glycolysis of the sarcoma, but inhibited its anaerobic glycolysis to the same degree as in normal tissues.

Crystalline penicillin G (15,000 units/ml) had no effect on glycolysis, indicating that the inhibition of glycolysis is due to some other factor present in the impure preparation. It cannot yet be stated whether or not the glycolysis-inhibiting factor is identical with the factor responsible for the protection of mice against endotoxins.

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IN THE LABORATORY

Benzene-Ether Extracted Rabies Vaccine

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In 1946–47 DeBoer and Cox (*1*) described a method for the preparation of complement-fixing antigens from brain by benzene (C_6H_6) extraction. Espana and Hammon (*2*) confirmed the validity of the principle and slightly modified the method of extraction. It occurred to us that the procedure might be adapted to the removal of lipid from brain tissue, as an initial step in the purification of rabies vaccine. Previously we had attempted by various common procedures to separate rabies virus from tissue elements. In some instances it seemed that the lipids of the brain may have interfered with sharp separation. In view of the results obtained by DeBoer and Cox, we attempted the preparation of vaccines by extracting lipids with various solvents.

Earlier experiences with the exposure of rabies virus to ether revealed that the virus is quite labile to ether when wet. Consequently, the first step was drying of the homogenized, infected, brain tissue from a frozen state under vacuum. Various lipid solvents were then added to the dry tissue, the volumes in each case being about twice the wet volume of the original tissue suspension. After a period of about 2 hrs in the cold room, the solvent was removed either by centrifugation and decanting or by filtration through a sintered glass filter of "C" porosity. In either case the sediment (or residue) was resuspended in the solvent and immediately recentrifuged or refiltered. Following the extration with benzene or other solvent, ether [$(C_2H_5)_2O$] extraction was done twice in a similar way but using ether at – 50° C. The tissue was then freed of the small amount of residual ether by placing the container in a jar and exhausting by water suction. Rehydration was accomplished simply by shaking with water and churning in a Waring blendor. The tissue suspensions were stored