It is of interest to note that tissues from a noncarcinomatous area in a carcinomatous breast were faintly stained, and that uninvolved skin from the same breast showed some reduction, while fibroadenomatous breast and skin from an appendectomy incision showed no color.

The application of the tetrazolium salts as intravital dyes is further suggested by the following observations. A gauze pack saturated with 1% tetrazolium chloride was applied to an ulcer surface which developed after postoperative irradiation of a carcinoma of the anus. It was not possible to determine by inspection whether the ulcer was due to recurrence of the carcinoma or to a late radiation necrosis. After 12 min the ulcer surface was stained bright scarlet-red, as was the gauze surface in contact with it. Removal of the ulcer and surrounding tissue revealed that the staining was limited to the surface of the ulcer to a depth of 2 mm below the surface. Frozen sections showed the presence of squamous cell carcinoma. A similar observation was made on an ulcer of the tongue. A cotton applicator saturated with 1% tetrazolium chloride was held on the ulcer for 10 min. The ulcer surface and the applicator were stained scarlet. Excision of the tongue showed the lesion to be a squamous cell carcinoma. Granulation tissue covering a third-degree burn ulcer was treated similarly for 20 min, but no color developed.

There is a suggestion that the tetrazolium compound is reduced more rapidly than by normal tissues, in regions where a local deficiency of oxygen exists. A leg was amputated for arteriosclerotic ischemia. Skin, deep fascia, and gastrocnemius muscle were taken from the fresh leg at the level of the knee joint and immersed in 1% tetrazolium chloride solution for 20 min at 37° C. No color developed. Similar skin, deep fascia, and muscle were taken from just above the internal malleolus of the tibia, 2″ above a gangrenous ulcer, and also immersed and incubated. The muscle and fascia showed no color although the skin was colored a faint pink, visible through the epidermis. On section it was demonstrated that the superficial layers of the epidermis were not colored. The cutis vera was a moderate pink color, and a fine scarlet line outlined the probable course of the basal layer of the epidermis.

These observations indicate that tetrazolium compounds may be used as a tool in the study of the intracellular metabolism of tissue anoxia. The scarlet-red color which develops on reduction is not ideal, since some mammalian tissues are only a different red. Work is under way not only to synthesize tetrazolium salts which may give formazans of other colors, preferably blue or green, but also to determine the fate of the tetrazolium chloride and to study the enzyme system which affects the rate of reduction in the organism. It is possible that a soluble substance which can be precipitated in differential quantities in some neoplastic tissues may prove a useful agent in the study and treatment of neoplasms.

References

1. COTTRELL, H. J. *Nature, Lond.*, 1947, **159**, 748.
2. DUTCHER, R. A. Report of Interrogation of Research Workers at the Agricultural High School at Hohenheim, September 21, 1945. (Technical Industrial Intelligence Branch, Joint Intelligence Service.)
3. KUHN, R., and JERCHEL, D. *Ber. dtsch. chem. Ges.*, **1941**, **74B**, 941, 949.
4. LAKON, G. *Ber. dtsch. bot. Ges.*, 1942, **60**, 299, 434.
5. MATTSON, M. A., JENSEN, O. C., and DUTCHER, R. A. *Science*, 1947, **106**, 294–295.
6. PECHMAN, H. V., and RUNGE, P. *Ber. dtsch. chem. Ges.*, 1894, **27**, 2920.
7. PORTER, H. R., DURRELL, MARY, and ROMM, H. J. *Plant Physiol.*, 1947, **22**, 149.
8. WARBURG, O., and CHRISTIAN, W. *Chem. Zbl.*, 1943, **114**, 1638.
9. WAUGH, T. D. *Science*, 1948, **107**, 275.

Palynological Studies at Sodon Lake: I. Size-Frequency Study of Fossil Spruce Pollen[1]

STANLEY A. CAIN

Cranbrook Institute of Science, Bloomfield Hills, Michigan

In another paper of this series Cain and Slater (*3*) are reporting a pollen analytical study of a 24′ profile of the peat and marl sediments of Sodon Lake; and Cain and Cain (*2*) have made a size-frequency study of the fossil pollen grains at Sodon Lake in relation to the modern species, showing something of the historical successional relationships of *Pinus Banksiana*, *P. resinosa*, and *P. Strobus*. The present paper considers the question of whether it is possible to identify the species of *Picea* which contributed to the fossil pollen of the blue clay layer of the 24′ level at the bottom of the profile.

The vegetational history of Sodon Lake, Oakland County, Michigan, in so far as it is revealed by the stratigraphic column, commences with the pre-Boreal spruce-dominated pollen spectrum referable to Period I in the scheme of Sears (*7*). This is the only level at which spruce is dominant. Of the 322 grains of this level, 85.7% were recognized as spruce, and about 100 of these were in a sufficiently good state of preservation and were oriented properly for measurement of the grain size. Grains seen squarely at right angles to their long axis (in dorsal, ventral, or lateral view) were measured across the maximum dimension of the grain, within the exine. This position is often along a line connecting the points of dorsal insertion of the bladders. The grains were found to range between 61.6 and 97.6 μ. The number of measurable grains was insufficient to produce a smooth size-frequency curve (Fig. 1, heavy-line curve) or to indicate surely whether one or more species of *Picea* were involved in the sedimentation.

[1] In the "Pollen Analysis Circular" Dr. Antevs posed the question: "Is pollen analysis the proper name for the study of pollen and its applications?" In No. 8 of the circular (page 6, October 1944) H. A. Hyde and D. A. Williams, of Wales, suggested the term *palynology* (from Greek *paluno*, to strew or sprinkle; cf. *pale*, fine meal, cognate with Latin *pollen*, flour, dust): the study of pollen and other spores and their dispersal, and applications thereof. Erdtman (1947) has accepted the term. Since it seems appropriate, we are using it formally for a series of papers somewhat broader in scope than those formerly included under "pollen analysis."

The next step was to prepare pollen from available contemporaneous material of the black spruce (*Picea mariana*) and the white spruce (*P. canadensis* or *P. glauca*) and to establish size-frequency curves and suitable statistics for the species. These species were selected because they are the ones present in Michigan today (see the map, Fig. 2). Fig. 1 shows size-frequency curves obtained for black spruce pollen (herbarium specimen from Isle Royale, Michigan) and for white spruce (herbarium specimen from Charlevoix, Michigan). The results were disappointing, for the black spruce, which occurs even today on bogs within Oakland County, Michigan, within a few miles of Sodon Lake, is obviously much smaller than most of the fossil grains, its curve overlapping the size-frequency distribution for fossil grains only slightly. The larger pollen grains of the white spruce essentially cover the whole size range of the fossil grains, but the modal size of the distribution suggests that this species probably did not contribute more than half of the fossil grains.

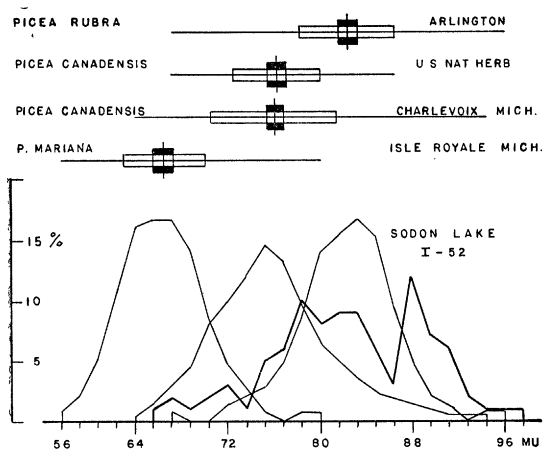

FIG. 1. Size-frequency and other statistical data for pollen grains of three species of spruce, and for fossil grains at Sodon Lake.

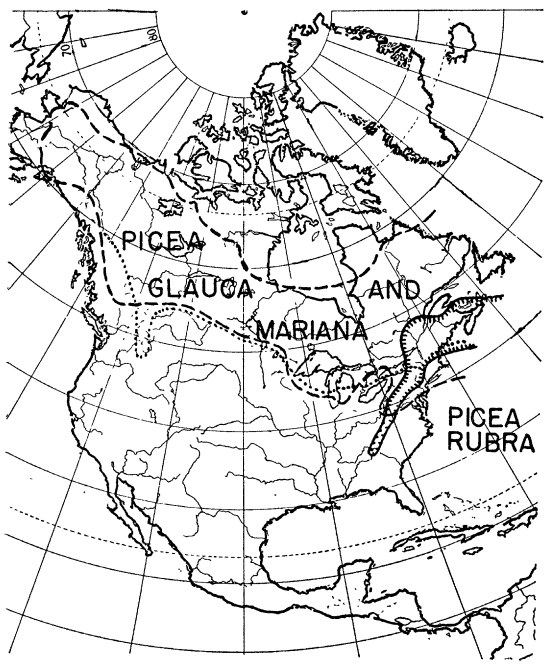

FIG. 2. Approximate areas of *Picea mariana* (Mill.) B.S.P., *P. glauca* (Moench) Voss, and *P. rubra* Link.

After speculating about this difficulty, the writer decided to compare the size-frequency characteristics of the eastern red spruce (*P. rubra*) with the fossil grains.

TABLE 1

SIZE-FREQUENCY CHARACTERISTICS OF *Picea* POLLEN

Size class (ocular lines; 1 line = 1.6 μ)	*P. mariana* Isle Royale, Michigan	*P. canadensis* (*glauca*) Pollen from U.S.N.H.	*P. canadensis* (*glauca*) Charlevoix County, Michigan	*P. rubra* Arlington Chemical Co.	Fossil *Picea* pollen Sodon Lake pre-Boreal period
36	0.7				
37	2.1				
38	5.3				
39	10.7				
40	16.1		0.5		
41	16.6		1.5		1.0
42	16.6	1.3	3.0	0.7	2.1
43	14.0	2.6	4.5	..	1.0
44	8.6	4.7	8.0	..	2.1
45	4.7	9.3	10.0	1.3	3.1
46	2.6	13.3	12.0	2.1	1.0
47	0.7	18.0	14.5	2.6	5.2
48	..	17.3	13.0	4.7	6.2
49	0.7	12.0	9.5	8.6	10.4
50	0.7	8.6	6.5	14.0	8.3
51		6.0	5.0	15.4	9.4
52		4.7	3.5	16.6	9.4
53		1.3	2.5	15.4	6.2
54		0.7	2.0	9.3	3.1
55			1.5	4.7	12.5
56			1.0	2.1	7.3
57			0.5	1.3	6.2
58			0.5	..	2.1
59			0.5	0.7	1.0
60				0.7	1.0
61					1.0
N	150	150	200	150	96
$\overline{X}$	66.86 μ	76.70 μ	76.54 μ	82.80 μ	..
σ	3.86 μ	3.82 μ	5.43 μ	4.25 μ	..
$PE_{\overline{x}}$	0.21 μ	0.21 μ	0.26 μ	0.23 μ	..
$2\frac{\sigma}{\sqrt{N}}$	1.01 μ	1.00 μ	1.22 μ	1.12 μ	..

Students of fossil pollen in the western Great Lakes area have never considered the possible presence of more than the two presently native species of the area. Most students have only designated the genus, but Wilson (*8*), Wilson and Kosanke (*9*), Wilson and Webster (*10*), and Friesner and Potzger (*5*) have named *Picea mariana* and *P. glauca* from sediments they have studied, without, however, publishing adequate statistical or other diagnostic characters. Not having studied these characteristics of the red spruce before, the writer was both surprised and pleased to find that at least the one collection of pollen available showed the species to have grains

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

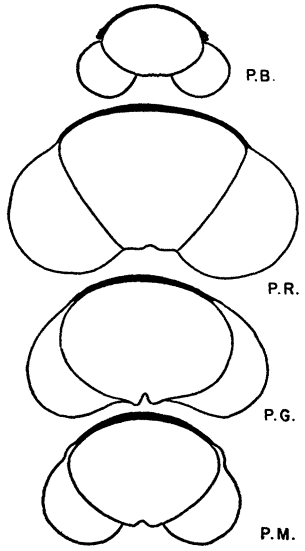

FIG. 3. Median optical views of pollen grains of the $\overline{X}$ size class for each species. (P. B.) *Pinus Banksiana* (45 μ), added for comparison with the spruces, (P. R.) *Picea rubra* (83 μ), (P. G.) *P. glauca* (77 μ), (P. M.) *P. mariana* (67 μ).

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.

References

1. CAIN, S. A., and CAIN, L. G. *Ecology,* 1944, **25**, 229.
2. CAIN, S. A., and CAIN, L. G., in press.
3. CAIN, S. A., and SLATER, J. V., in press.
4. DICE, L. R., and LERAAS, H. J. *Contr. Lab. vert. Gen., Univ. Mich.,* 1936, **3**, 1.
5. FRIESNER, R. C., and POTZGER, J. E. *Butler Univ. bot. Stud.,* 1946, **8**, 24.
6. MUNNS, E. N. *U. S. Dept. Agric. Misc. Publ.,* 1938, **287**, 1.
7. SEARS, P. B. *Bot. Gaz.,* 1942a, **103**, 751; *Amer. J. Bot.,* 1942b, **29**, 684.
8. WILSON, L. R. *Rhodora,* 1938, **40**, 137.
9. WILSON, L. R., and KOSANKE, R. M. *Torreya,* 1940, **40**, 1.
10. WILSON, L. R., and WEBSTER, R. M. *Ia. Acad. Sci.,* 1943, **50**, 261; *Trans. Wis. Acad. Sci., Arts, Lett.,* 1942, **34**, 177.

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

ERNEST KUN

Departments of Pharmacology and *Medicine, University of Chicago*

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.