be explained. Lactic dehydrogenase activity was measured in homogenates of various tissues from an untreated control F1 mouse and an F1 mouse that had been inoculated with leukemia 82B 16 days previously. The SLD of the control mouse was 760 units and that of the leukemic mouse 60,000 units. Table 2 compares the LD activity per gram of wet tissue in these two mice. The serum levels present the most striking differences between the leukemic and nonleukemic mouse. A comparison of the LD activity of the other tissues reveals higher values in the homogenates of the viscera of the leukemic mouse, although the differences are not as impressive as those observed in the serum.

The observations on various mouse leukemias reported here indicate that mouse leukemias, like some human leukemias, result in an increase in SLD. The mechanism of the increased serum activity is unknown.

CHARLOTTE FRIEND
FELIX WRÓBLEWSKI
Sloan Kettering Institute and
Department of Medicine,
Memorial Center, New York

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2. This work was supported in part by a grant from the National Cancer Institute, U.S. Public Health Service; the American Cancer Society; and the Damon Runyon Memorial Fund for Cancer Research. We wish to acknowledge the technical assistance of Herman Steiniger.

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Evolution of Respiratory Enzyme System during Germination of Bacillus subtilis

Church and Halvorson (*1*) reported that intact spores of *Bacillus cereus,* following activation by heat, oxidize glucose, gluconate, 2-ketogluconate, and pyruvate, but fail to oxidize glucose-6-phosphate, fructose-6-phosphate, hexose diphosphate, acetate, ribose, or fructose. On the other hand, cell-free extracts of spores of the same species which have been prepared by sonic oscillation or by grinding oxidize glucose and pyruvate but not gluconate, 2-ketogluconate, glucose-6-phosphate, fructose-6-phosphate, or hexose diphosphate. Hardwick and Foster (*2*), after working with cell-free extracts of spores from several aerobic bacteria, reported an absence of any measurable respiratory enzymes.

In a previous report (*3*), we showed that spores of *Bacillus subtilis* (PCI 219) develop into vegetative forms after passing through four separate phases—namely, resting spores, appearance of translucent area in spores, formation of germ pores, and emergence of a new bacillus. In this report, it is observed that respiratory-enzyme systems are formed according to the phase of development of spore germination and that respiratory-enzyme systems of germinating spores differ from those present in vegetative forms.

Spores of *B. subtilis* (PCI 219) were harvested from a 5-day culture grown on a meat-extract agar medium at 37°C, heated at 80°C for 20 minutes, and then washed in distilled water three times and resuspended in distilled water. The spores of this suspension were used as resting spores for the experiment. Oxygen uptake by spores was measured in a Warburg manometer at 37°C.

In the presence of L-asparagine, the resting spores showed an increased uptake of oxygen when caramelized glucose was used as the substrate instead of the unheated glucose. It was previously reported (*4*) that a significant number of spores of *B. subtilis* (PCI 219) will change to the translucent spore stage from the resting stage if the phosphate-buffer solution contains a caramelized sugar (glucose, fructose, maltose, lactose, or sucrose) instead of unheated sugar with one of the amino acids (L-asparagine, DL-isoleucine, DL-serine, or DL-valine).

It is apparent that the factors which favor translucent-spore formation as previously reported (*4*) also result in an increased oxygen uptake by the resting spores. In other words, resting spores change to the translucent spores in a phosphate buffer that contains caramelized glucose and L-asparagine; they also show an oxygen uptake. On the other hand, almost none of the resting spores in a phosphate buffer that contains unheated glucose and L-asparagine change to the translucent forms or show a measurable oxygen uptake. An examination to determine whether translucent spores are able to oxidize unheated glucose was therefore conducted.

Translucent spores were prepared by incubating the resting spores in the phosphate solution containing caramelized glucose (1 percent) and L-asparagine (1.5 mg/ml) for 1 hour at 37°C. These translucent spores were washed in distilled water three times and resuspended in distilled water. Manometric data are given in Fig. 1, where results clearly indicate that the translucent spores rapidly oxidize unheated glucose.

To determine the pathways of oxidation of glucose by the translucent spores, two experiments were performed. In one, some intermediates of glycolysis and of the tricarboxylic acid cycle were used as substrates, and, in the other, some inhibitors were used. Manometric data from these experiments are give in Table 1. These data indicate that the translucent spores do not oxidize fructose-1, 6-diphosphate, pyruvate, citrate, succinate, or malate and that the oxidation of glucose is not inhibited by HCN or NaF. These facts show that glucose oxidation by the translucent spores may not proceed on the pathways of Embden-Meyerhof and the tricarboxylic acid cycle.

Table 1. Oxygen uptake by the translucent spores in the presence of various intermediates of the tricarboxylic acid cycle and the Embden-Meyerhof pathway. The Warburg vessels contained 0.5 ml of translucent spore suspension, 0.2 ml of substrate, 0.1 ml of inhibitor, and 1.5 ml of 0.1*M* phosphate buffer at *p*H 7.2 and 37°C in a fluid volume of 3 ml.

Substrate	Inhibitor	QO_2 (*N*)
0.1*M* Glucose		138.84
0.1*M* Malate		42.65
0.1*M* Pyruvate		44.47
0.1*M* Succinate		42.22
0.1*M* Citrate		38.12
0.1*M* Fructose-1, 6-diphosphate		61.41
Endogenous		39.53
0.1*M* Glucose	0.01*M* HCN	139.14
0.1*M* Glucose	0.01*M* NaF	151.80

Vegetative forms of *B. subtilis* are able to oxidize glucose-6-phosphate, fructose-1,6-diphosphate, pyruvate, malate, or succinate. Moreover, these oxidations are inhibited by HCN and NaF. It seems, therefore, that glucose oxidation in translucent spores differs from that in vegetative forms.

To determine the pathways of glucose oxidation by the translucent spore, we used sodium gluconate as a substrate first. The results are shown in Fig. 1. It is clear

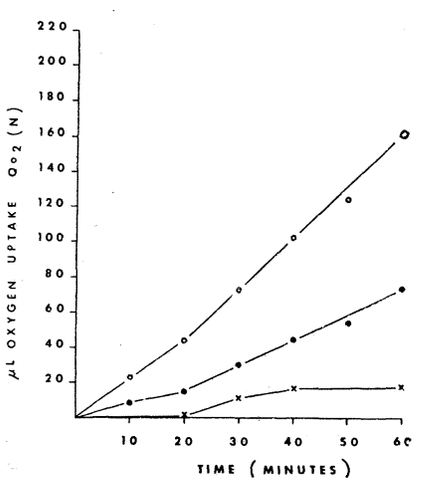

Fig. 1. Unheated glucose and sodium gluconate oxidation by translucent spores: ○—○, glucose; ●—●, gluconate; ×—×, endogenous. The Warburg vessels contained 0.5 ml of translucent spore suspension, 0.2 ml of substrate, and 1.5 ml of 0.1*M* phosphate buffer at *p*H 7.2 and 37°C in a fluid volume of 3 ml.

from these data that gluconate is oxidized by the translucent spores. There are two known ways by which glucose is oxidized to gluconic acid. One is the Warburg-Dickens-Lipmann-Horecker pathway (*5*) and the other is the Stokes-Campbell route (*5*). The former requires the presence of phosphoric acid, but the latter does not. We found that glucose and gluconate oxidation proceeded in veronal buffer, which is phosphate-free, as efficiently as in phophate buffer. This would eliminate the Warburg-Dickens-Lipmann-Horecker pathway.

It is interesting to note that vegetative forms of this species have the cytochrome system and other oxidative enzymes (*6*), whereas the germinating spores can oxidize glucose or gluconate only in a phosphate-free buffer.

From this fact it may be assumed that the glucose oxidative enzyme system of *B. subtilis* (PCI 219) may develop in a stepwise manner as germination proceeds and as the cells change into the vegetative form.

Y. Hachisuka*, N. Asano,
M. Kaneko, T. Kanbe
Department of Bacteriology,
Nagoya University School of Medicine,
Nagoya, Japan

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* Present address: University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston.

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Rapid Ion-Exchange Method for Determining Total Fluoride in Impure KF · HF

As a result of the continuing need for large numbers of control analyses on electrolyte for fluorine generation cells at this plant, it became necessary to develop a rapid method of analyzing potassium acid fluoride (KF · HF) for total fluoride content (*1*). Accuracy comparable to that of the photofluorimetric method (2), which has been in use, was required. Consequently, an ion-exchange technique was investigated, and a procedure was developed, modifying the general methods described by Honda (*3*) and Roper et al. (*4*), in which the cations are removed from the solution, and the fluoride-containing eluant is titrated with NaOH.

A sulfonated hydrocarbon cation exchange resin (Rohm and Haas Amberlite IR-120) supported on 100-mesh nickel screen is used in the simple apparatus shown in Fig. 1. The resin is first activated by washing with 1 lit of 2-percent H_2SO_4 at 30ml/min., the acid that remains in the tube being washed out with distilled water. The weighed electrolyte sample (impure KF · HF) is dissolved in distilled water, and an aliquot of about 10 ml is passed through the ion-exchange column into a 400-ml polyethylene beaker. The column is then washed with distilled water until there is about 275 ml of eluate. This solution is then titrated to a phenolphthalein endpoint with NaOH solution prepared from CO_2-free NaOH and standardized at approximately 0.1*N* with NaF.

Samples of known amounts of reagent-grade NaF which were dried at 110°C for 24 hours were used to check the method prior to applying it to samples of KF · HF electrolyte. Good results, which are shown in Table 1, were obtained by using the procedure outlined here. As a further check, this ion-exchange procedure was directly compared against the photofluorimetric technique previously used by analyzing parts of the same electrolyte sample (impure KF · HF) by both methods. The portion for photofluorimetric analysis was steam-distilled and titrated with thorium nitrate in a modified Klett fluorimeter, using morin as the indicator; the portion for ion-exchange analysis was handled as described in preceding paragraphs. Results, in terms of percentage of fluoride found, are shown in Table 2.

The values obtained, together with data subsequently obtained in routine use of this procedure, show that it yields results of accuracy comparable to that obtained by the photofluorimetric method in about one-fourth of the previously required time. In general, the titration is correct to 1 or 2 drops with phenolphthalein as the indicator. Simple checks showed that accuracy was not affected by varying the rate of flow of sample and wash water in the column between 5 and 30 ml per minute or by varying the depth of resin in the column from 6 in. to 12 in. It was found, however, that the higher rates of flow with a 6-in. resin bed gave slightly erratic results. A single 12-in. bed has been used intermittently for 3 weeks (approximately 50 determinations) without regeneration. Since regeneration is easily accomplished with 2-percent H_2SO_4, the resin may be used indefinitely.

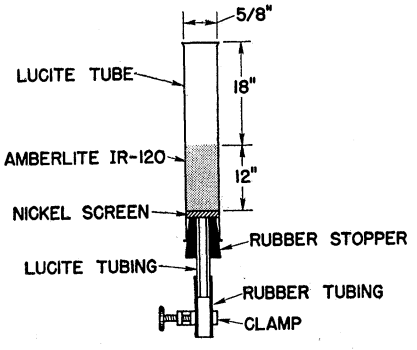

Fig. 1. Ion-exchange column.

Table 1. Results of analyses of samples of known fluoride content.

Sample	Fluoride present (mg)	Fluoride found (mg)
1	63.34	63.67
2	55.87	55.87
3	57.23	57.43
4	58.54	58.53
5	55.69	55.61
6	50.80	50.80
7	47.55	47.54
8	47.14	47.08
Avg.	54.52	54.57

Table 2. Comparison of percentage of fluoride found by ion-exchange and photofluorimetric methods in impure KF · HF samples.

Sample	Photo-fluorimetric method	Ion-exchange method
1	58.81	58.73
2	58.85	58.72
3	58.80	58.77
4	58.86	58.80
Avg.	58.83	58.76

F. A. Koehler
W. A. Levier
Special Analysis Department, Technical Division, Goodyear Atomic Corporation, Portsmouth, Ohio

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Ratio of Ionium to Uranium in Coral Limestone

Samples of coral limestone cuttings taken over the first 200 feet in drilling on Elugelab Island during the summer of 1952 (*1*) have been analyzed for uranium by the sodium fluoride fluorometric method and for ionium by a radiochemical procedure which involves carrier-free separation of the total thorium in the