of a great variety of unconnected forces, can be described by the simplest mathematical function, x^n and e^x.''

TABLE 1

Sizes of glacial boulders

Boulder length (feet)	James River area*	Crosby-Minot area†
4.0 to 4.4	615	825
4.5 " 4.9	377	355
5.0 " 5.4	232	212
5.5 " 5.9	121	142
6.0 " 6.4	103	83
6.5 " 6.9	55	51
7.0 " 7.4	34	27
7.5 " 7.9	21	23
8.0 " 8.4	20	14
8.5 " 8.9	4	7
9.0	8	
9.1		4
9.2		1
9.3		1
9.5	6	5
9.6	1	
10.0	7	1
10.2	1	
10.5	2	3
10.7	1	
10.8		1
11.0	1	
11.2		3
11.3		1
11.6		1
12.0	1	
13.0	2	1
13.5	1	1
15.0	1	
17.0	1	1
Totals	1,615	1,763

Rock types of glacial boulders

	James River area		Crosby-Minot area	
	Number of boulders	Percent of total	Number of boulders	Percent of total
Granite	1,481	91.7	1,084	61.5
Granitic gneiss	54	3.3	476	27.0
Very hard hornblende-biotite gneiss	40	2.5	43	2.4
Garnet schist			14	0.8
Paleozoic limestone	30	1.8	99	5.6
Very hard siliceous sandstone			32	1.8
Basic igneous rock	1	0.7	8	0.9
Greenstone	7			
Tertiary sandstone (Fort Union form)	2		7	
Totals	1,615	100.0	1,763	100.0

* James River area, 1,170 square miles examined; average, 1 boulder to 0.73 square mile.

† Crosby-Minot area, 2,600 square miles examined; average, 1 boulder to 1.47 square miles.

More than 90% of the large boulders in each area are of granite, gneiss, and schist, and nearly all are of hard, unweathered rock. No large weathered boulders were seen in the Crosby-Minot area, but 10 large boulders of deeply weathered granite were recorded in the James River area. Table 1 shows the number of boulders of each size-group and the kinds of rock in each area.

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Characteristics of the Desoxycholate-treated Cytochrome Oxidase[1]

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In 1947 the authors reported the preparation and partial purification of a solubilized cytochrome oxidase (*9*). This article is concerned with its further characteristics and the methods employed in an attempt to purify the desoxycholate-treated cytochrome oxidase.

Physical appearance: The insoluble cytochrome oxidase complex (*1, 5*) is tan, opaque, and particulate in appearance. A partially purified preparation (2–3%), made as previously described (*10*), is clear to the naked eye and light yellow in color.

Lyophilization: All of the desoxycholate-treated oxidase preparations reported in this and in previous papers may be lyophilized from the frozen state and stored at 0° without loss of activity.

Variations in Q_{O_2} protein: The partially purified preparations from lamb heart vary in their activity by as much as 35%, having Q_{O_2} protein values ranging from 1,500 to above 2,000 when tested with the hydroquinone system previously described (*10*). These variations may be ascribed in part, at least, to the amount of protein in the insoluble cytochrome oxidase suspension which seems to be directly related to the amount of sodium desoxycholate that must be used for the first extraction.

Gel formation: The preparation of the partially purified enzyme is often made difficult by the formation of a gel. This gelation seems to be a property of the desoxycholate when dissolved in phosphate buffer (*7, 8*). The oxidase activity is not impaired, however. If the final supernatant is left to stand at 0°, it will invariably gel. This gel may be liquefied by simply warming the tube in the hand.

Denaturation on standing: A partially purified preparation (1.5–3%) has been kept at 0° in both the con-

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centrated form (gelled) and diluted form for 6 days without loss of activity. When tested on the tenth day, after bacterial contamination was marked, the concentrated preparation showed no loss in activity and the dilute preparation a 30% loss.

In view of the loss in activity of the insoluble cytochrome oxidase complex when tested in a system involving the oxidation of D-glucose by added D-glucose dehydrogenase protein, diphosphopyridine nucleotide, and cytochrome C (*3*), it is particularly interesting to note that the insoluble oxidase preparations discussed above showed no significant loss in activity in 13 days when tested with the hydroquinone system. These observations suggest that a factor is destroyed in the aging of the insoluble cytochrome oxidase complex which is necessary for the oxidation of D-glucose by the previously mentioned system, but which is not required for the oxidation of hydroquinone.

TABLE 1

Temperature (°C)	Q_{O_2} protein	Loss in activity (%)
Unheated	2180	...
40	1150	53
45	710	67
50	460	79
55	150	93
60	0	100

Heat stability: Table 1 shows the destruction of enzymatic activity at a relatively low temperature. There was no visible precipitate in any of the tubes after the partially purified and diluted (1: 25) preparation (2–3%) had been heated for 5 min at the temperatures noted. The tests were made as previously indicated (*10*), except that semicarbazide (0.3 ml of a 0.1 M solution) was used as a ketone fixative.

In another experiment, neither the insoluble nor the partially purified preparation suffered any loss in activity after being heated for ½ hour at 37°.

pH optimum: Preliminary experiments indicate that the oxidation of reduced cytochrome C by a partially purified, lyophilized preparation (2–3%) occurs most rapidly at pH 7. The 0.05 M buffers used and their pH values are as follows: acetate, pH 5.04; phosphate, pH 7.0; phosphate, pH 7.95; borate, pH 8.8; glycine, pH 11.05.

The methods used in an attempt to purify the oxidase are as follows:

Dialysis: Dialysis for 18 or 24 hrs does not significantly alter the activity. However, in an experiment where a 1.5–3% preparation was used in both the concentrated and the dilute (1: 25) form, there was a considerable precipitate at the end of 3 days of dialysis (the water being changed each day) and the preparation was inactive. A crude test showed little or no desoxycholate to be present. Added desoxycholate dissolved the precipitate but did not restore the activity.

Other experiments indicate that it may be possible to remove the desoxycholate without too great a loss in activity by dialyzing against 0.01 M KH_2PO_4–Na_2HPO_4 buffer of pH 7.4 or above. This is being further investigated.

Centrifugation: In 1944, Haas (*4*) reported the separation of his cytochrome oxidase preparation into 2 components by centrifugation at 10,000 rpm for 2 hrs. This separation has been reviewed by Keilin and Hartree (*6*). Our experiments demonstrate that even the dilute preparations (1: 25) can be centrifuged at 20,000 × g for 2 hrs without any indication of a separation of components.

Effect of pH: The pH of our partially purified preparation (1.5–2%) is approximately 8. By gradually lowering the pH of a diluted sample with HCl, we find that at pH 6.2 the activity is still in the supernatant (slight precipitate removed), while at pH 5.78 the partially inactivated enzymes are in the precipitate together with the desoxycholate.

Use of alcohol: A 0–4% preparation can be partially purified by fractional precipitation with ethyl alcohol. Preliminary experiments on a lyophilized, partially purified preparation (1.5–2%) show that no visible precipitate forms with as much as 80% of alcohol at 0°. This concentration of alcohol completely destroys the oxidase.

Effect of ether: It was assumed, as previously suggested (*10*), that the oxidase complex might be a lipoprotein. An attempt was made to separate the lipoidal component by the use of ethyl ether. The activity of a partially purified oxidase (1.5–2%) was completely destroyed by washing with ether.

Preferential solubility in sodium desoxycholate: It has been shown (*10*) that the addition of a small amount of desoxycholate (1.5 or 2%) to the insoluble preparation dissolves proteins of low activity, whereas larger amounts of desoxycholate (3 or 4%) dissolve active proteins as well. It was on this observed differential solubility of the active and inactive proteins that the partial purification was based. It seemed possible also that the various components of the oxidase complex could be separated by successive additions of small amounts of desoxycholate. Favorable results have been obtained by the use of this method, and a preliminary report has been presented (*2*).

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