characterized by no change in cardiac output, less bradycardia than in the pressor phase, and a decrease in peripheral resistance. 2. When tetrahydrozoline was administered orally, no pressor response was noted. Twenty-seven of 34 patients exhibited an average reduction of 44 mm Hg systolic pressure, 25 mm Hg diastolic pressure, and an average decrease in heart rate of 20 beats per minute. Tolerance usually developed in 7 to 10 weeks.

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Human Liver Succinoxidase.* (22952)

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This report deals with conditions necessary for maximum succinoxidase activity in human liver homogenates, the distribution of succinoxidase in subcellular components of human liver and the range of normal for whole homogenates of human liver when "standard conditions" are used(1) and when optimum conditions are used. Waterlow(2), using the Cartesian diver technic, found one-tenth the activity obtained during the present study. Davis *et al.*(3) recently reported succinoxidase Qo_2/mg (dry weight) of slices. The thickness of the slices was not indicated. The incubation medium was not specified beyond saying that Krebs-Ringers succinate was used. A "standard technic" is referred to(1), but this gives only a method for homogenates of small animal tissues and not for human liver slices. How important is it to specify the exact conditions used for measuring succinoxidase activity or to use optimum conditions? What can be considered a normal level of activity? These are questions which led to the following work.

Materials and methods. The selection of patients for biopsy and the preparation of tissue have been described(4). 'Sediment' as used below indicates all particulate matter in a whole homogenate which is sedimentable at 10,000 × g for 30 minutes with one washing in isotonic sucrose. The reaction mixture used initially was that recommended by Umbreit(1). Standard manometric technics were used with an equilibration period of 10 minutes. Readings were taken for 70 minutes. The reaction was zero order for this period. As the optimum level of each constituent was determined this level was substituted in all subsequent experiments until a final optimum reaction mixture was obtained. Using these optimum conditions, variation of cytochrome c and substrate concentrations were restudied. The pH of the reaction mixtures was determined with a Beckman model G pH meter before and immediately after the experiment was completed. The cytochrome c† and sodium succinate were commercial preparations. Ascorbic acid was used for determination of cytochrome oxidase activity. The manometric analyses, including controls for endogenous respiration, were carried out at least in duplicate. The average endogen-

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† Standardization of cytochrome c was spectrophotometrically(1). For an assumed molecular weight of 13,000, various shipments of cytochrome c from Nutritional Biochemicals Co., Cleveland, O., proved to be from 52 to 60% pure. This material gave the same optimum concentration of cytochrome c for rat liver succinoxidase as has been reported(5).

TABLE I. A Fractionation of Human Liver Q_{O_2} (N) Succinate.*

Fractions†	Sedimentation force × g	Min.	Washing	Enzyme activity, μl/ml, 5% homog./hr	Recovery of enzyme,‡ %	Total nitrogen,§ mg	Q_{O_2} (N)
Whole homogenate				192.20	100	2.755	139.5
Nuclear	211	10	1	14.20	7.39	.587	48.4
I	1,035	10	0	66.35	34.40	.380	349.21
II	1,800	10	"	26.52	13.80	.160	331.5
III	3,200	10	"	23.25	12.10	.115	404.3
IV	5,000	10	"	8.81	4.58	.057	309.1
V	8,890	10	"	16.65	8.62	.048	693.7
VI	18,000	30	"	18.86	9.80	.231	163.2
Supernatant			"	13.77	7.17	1.026	26.84
Total recovery, %					97.86	94.51	

* Distribution of succinoxidase in homogenate of human liver prepared from a biopsy specimen.

† Reaction mixtures contained 1 ml of 0.1M phosphate buffer, pH 7.4, 0.5 ml of 5% whole homogenate or its equivalent of any other fraction, 0.1 ml of 0.5M sodium succinate, 0.1 ml of 12×10^{-2}M $CaCl_2$, 0.1 ml of 12×10^{-2} $AlCl_3$, 0.9 ml of 2.24×10^{-4}M cytochrome c for the whole homogenate and 0.6 ml of 2.24×10^{-4}M cytochrome c for fractions other than whole homogenate.

‡ Activity of fraction × 100 divided by activity of original homogenate.

§ Per ml of original (10%) homogenate or its equivalent.

ous respiration for all experiments was 4 μl/hour 0.5 ml of 5% homogenate. Total nitrogen was determined according to Koch and McMeekin(6). The details of fractionation are presented in Table I. Microscopically there was some contamination of the nuclear fraction by mitochondria and the first mitochrondrial fraction contained a few nuclei.

Results. Cytochrome c was the moiety investigated first, since it is known that the concentration of cytochrome c in human liver is only about one-fourth that of rat liver(7) and it was anticipated that more exogenous cytochrome c might be required than the 0.133×10^{-4} M recommended for rat liver (1). The whole homogenate of human liver had an increasing succinoxidase activity until the cytochrome c content had been increased to 0.67×10^{-4} M. Approximately 0.45×10^{-4} M cytochrome c is required for maximum activity in sediment (Fig. 1). Cytochrome oxidase was not a limiting factor in these experiments.

The requirement for substrate proved to be lower for human than for rat liver homogenate. The optimum substrate concentration in human liver was the same for both the homogenate and sediment (Fig. 2). An inhibition of activity appeared with increasing substrate concentration. This same effect was observed by Schneider and Potter(5). Attempts to purify the substrate by reprecipitation had no effect on this inhibition. Since oxygen consumption was observed to be linear with time at all substrate concentrations and since calcium was present in all flasks(8,9), it seems unlikely that an inhibitor such as oxalacetate was formed as a result of higher substrate concentrations. The optimum sub-

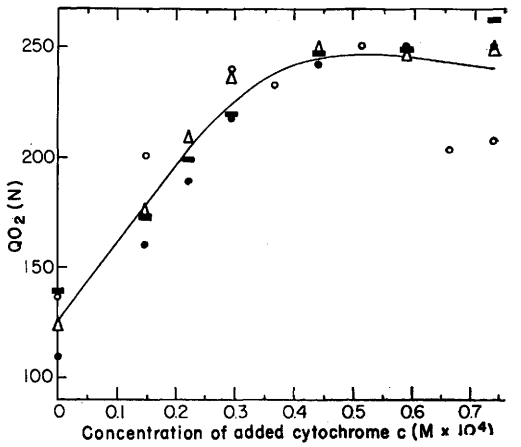

FIG. 1. Relationship of added cytochrome c to succinoxidase activity for sediment using liver biopsies from 4 patients. Sediment includes all particulate matter obtained by centrifugation at 18,000 × g for 30 min and washed once in 0.25 M sucrose.

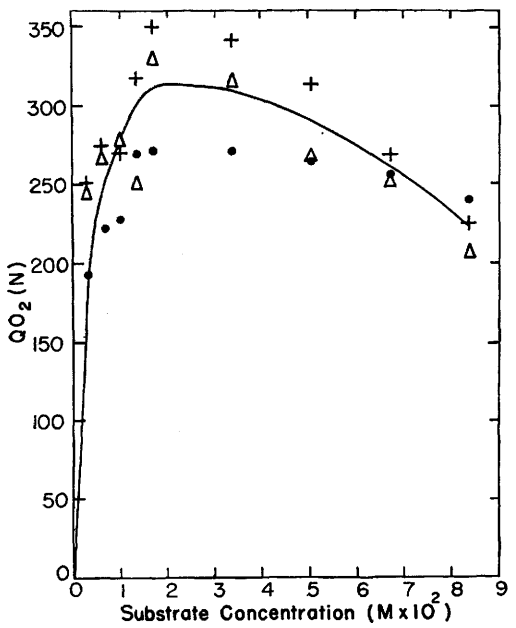

FIG. 2. Effect of substrate on succinoxidase in sediment, 3 exp.

strate concentration is around 1.6×10^{-2} M (Fig. 2). The optimum reported for homogenates of rat liver is 5×10^{-2} M(5).

Experiments in which $AlCl_3$ was held constant at 4×10^{-4} M and $CaCl_2$ varied, plus experiments with $CaCl_2$ maintained at 4×10^{-4} M and $AlCl_3$ varied, revealed optimum activity in all situations where at least 1.2×10^{-3} total cation was present. $FeCl_3$ gave equally satisfactory activity alone or in any combination with $CaCl_2$ and/or $AlCl_3$. pH optimum curves for whole homogenate demonstrated that pH 7.4 is to be recommended for human liver succinoxidase (Fig. 3). Table I shows the results of cell fractionation in a typical experiment. Table II presents data from normal appearing human liver whole homogenates and demonstrates the increase in "normal" succinoxidase activity that results from using optimum conditions for human liver instead of conditions originally worked out for rat liver(1).

Discussion. Succinoxidase in normal adult human liver has an activity of 150 to 200 Q_{O_2}/N as determined in whole homogenates under optimum conditions and a Q_{O_2}/N of 300 for the sediment from such homogenates (Table II). This is less than one-third the activity found in rat liver in our laboratory but is considerably higher than the activity that has been reported for normal infant liver using a different technic(2). The known low cytochrome c concentration of human liver plus the requirements for relatively high concentrations of added cytochrome c in homogenized and dilute samples suggests that there may be a more efficient spatial chemical organization in the cells of larger animals. The observations made regarding cation requirements support the thesis that they have a nonspecific effect only. The possibility that calcium is an essential cation in trace amounts is not excluded, however, since no attempt was made to remove the calcium which was already present in the liver. The inhibitory effect of substrate when present in above optimal concentrations has been observed in experiments with rat liver as well but no explanation has been found. The importance of using optimum conditions is illustrated in Table II. As work is done on human tissues repetition will be avoided and the work will be of greater value if conditions are detailed so that they can be reproduced.

Using optimum conditions a number of complete fractionations was carried out as illustrated by a typical experiment in Table I. It was observed that the greatest total concentration of succinoxidase is in the large

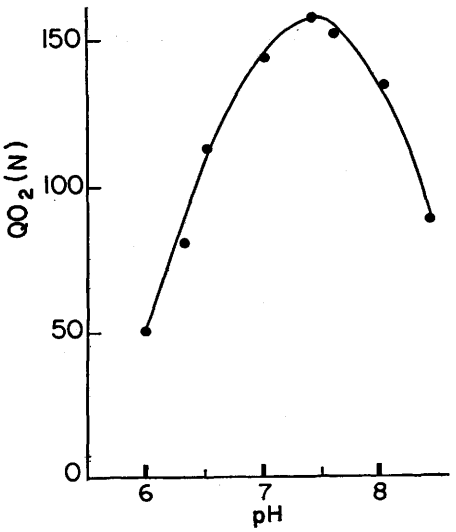

FIG. 3. Effect of pH on succinoxidase activity in whole homogenate.

TABLE II. Qo_2 (N) Succinate for a Series of Human Liver Homogenates Using the Standard Manometric Method (Umbreit) as Compared with a Later Series Using Optimum Conditions.

Method	No. of patients studied	Qo_2 (N) mean	S.D.†
Conditions recommended for rat liver(1)	6	76.4	8.8
Optimum conditions for human liver*	10	177.2	30.2

* See footnote to Table I.

† Stand. dev. $= \sqrt{\frac{\Sigma (x^2)}{N}}$.

granule fraction. The activity seen in the nuclear fraction can be explained by contamination with large mitochondria and likewise the small particles of fraction VI contain some mitochondrial material as well as most of the microsomes. The distribution of succinoxidase in subcellular particulate matter was observed to be similar in human liver to the distribution in rat liver and illustrates again the heterogeneity of mitochondria(10, 11). Succinoxidase is a better enzymatic identification of mitochondrial material in homogenates of human liver than fumarase, citrase or isocitrase(4).

Summary. The range of succinoxydase activity in whole homogenates of human liver is 150 to 200 Qo_2/N. The requirements for added cytochrome c are high in spite of a known low concentration of endogenous cytochrome c, suggesting a more efficient spatial chemical organization within the intact cells of larger animals. The distribution of succinoxidase in subcellular particles is shown. The requirements for optimum succinoxidase activity are indicated and it is demonstrated that the activity is more than doubled when optimum concentrations of flask contents are used as compared with concentrations originally developed for small animal tissue homogenates. Succinoxidase is a good enzymatic identification for mitochondria in studies on the chemistry and enzymatic activity of human liver cell fractions.

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