CLXXXVII. THE DISTRIBUTION OF THE SUCCINIC OXIDASE SYSTEM IN ANIMAL TISSUES

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IN recent work two functions have been suggested for the enzyme system which catalyses the oxidation of succinate to fumarate in many animal tissues. In muscle and in kidney the oxidation of succinate to fumarate appears to be a step in a cycle of reactions involved in the oxidative breakdown of lactate and pyruvate or of triose [Toenniessen & Brinkman, 1930; Elliott & Schroeder, 1934; Elliott *et al.* 1935; Elliott & Greig, 1937; Krebs, 1937]. According to Szent-Györgyi and his collaborators [e.g. Szent-Györgyi, 1935, 1937] the succinate-fumarate system occupies a central position in the system of oxidation-reduction catalysts which are responsible for tissue respiration, i.e. for the oxidation of cell metabolites in general. The enzymes concerned in succinate oxidation are known to be widely distributed and to occur in high activity in some tissues [Battelli & Stern, 1910]. However, in two types of tumour tissue which respired at a rate comparable with that of normal tissues, Elliott *et al.* [1935] found practically no succinate-oxidizing activity. It therefore seemed that useful information concerning the mechanism of tissue respiration would be obtained by a quantitative survey of the succinate-oxidizing activity and of the enzymic components responsible for succinate oxidation in various types of tissue. The results of such a survey are here presented.

It is generally accepted [Szent-Györgyi, 1924; Fleisch, 1924; Keilin, 1929] that the oxidation of succinate occurs under the influence of two enzymes and a "carrier". One of the enzymes, the dehydrogenase, is specific for succinate and under its influence succinate can be oxidized to fumarate while methylene blue, for instance, accepts H atoms and is reduced to leucomethylene blue. In ordinary circumstances, without methylene blue, the H acceptor is oxidized cytochrome. The cytochrome thus reduced acts as a H carrier and is reoxidized by molecular O_2 under the influence of the long known "indophenol oxidase" which Keilin [1929] has identified with the "respiratory ferment" of Warburg. Following the usual terminology for enzymes, this enzyme is best described as "cytochrome oxidase" since cytochrome is the only known substrate for it. *p*-Phenylene diamine and the "Nadi reagent" (dimethyl-*p*-phenylenediamine + α-naphthol) have been mentioned as substrates for this enzyme, but it seems clear (see below) that these substances also are oxidized through the mediation of cytochrome.

For the purpose of this paper the following terms will be used:

"Succinoxidase" will mean the entire aerobic succinate-oxidizing complex of oxidase, cytochrome and dehydrogenase.

"*p*-Phenylenediamine oxidase" will mean the *p*-phenylenediamine-oxidizing complex of oxidase and cytochrome.

"The oxidase" will mean cytochrome oxidase.

"The dehydrogenase" will mean succinic dehydrogenase. It seems certain that no separate coenzyme is involved in this dehydrogenase activity [Boyland & Boyland, 1934; Andersson, 1934].

Rosenthal [1937] has suggested that the determination of the rate of succinate oxidation in very thin slices of tissue could be used as a means of obtaining a minimum estimate of the respiratory ferment in intact tissue. While the determination of the activity of the succinoxidase in intact cells can best be determined in this way, it will be shown below that the full activity of the respiratory ferment is not displayed unless there is excess of the dehydrogenase and cytochrome present in the cells. Estimates of the full respiratory ferment activity of various tissues have been made by determination of the rate of *p*-phenylenediamine oxidation by broken up tissues in the presence of excess cytochrome.

Experimental

General methods

Except in a few specified experiments the tissues studied were prepared by "homogenization" of known weights of tissue in definite volumes of $M/10$ phosphate buffer, pH 7·4. The apparatus recently described by Potter & Elvehjem [1936], which reduces tissues rapidly and conveniently to a fine suspension which can be pipetted, was used. In most cases the tissue was chopped up with scissors whilst muscle was passed through a Latapie mincer, before homogenization. With some rather tough tissues the homogenization took several minutes and the homogenizer tube was kept cool in ice water. It was sometimes difficult to break up the last few pieces of tissue without continuing the homogenization for a long time; the weight of these pieces was determined and deducted from the total amount taken. Tissues from freshly killed animals were used; the suspension was kept in a refrigerator between experiments and it was used only during the day on which it was prepared. The suspensions contained amounts of moist tissue ranging from 50 mg. (heart, liver, kidney) to 200 mg. (spleen, tumours) per ml. With many tissues it was necessary to take material from a number of rats to obtain enough suspension for a set of experiments. In every experiment samples of the chopped tissue before homogenizing were weighed and dried at 100–110°, to determine the wet weight/dry weight ratio.

For the determination of O_2 uptake rates, simple Barcroft differential manometers were used [see Dixon, 1934]. The vessels contained air and a total of 3 ml. fluid, of which 1 ml. was $M/10$ phosphate buffer, pH 7·4, added separately or with tissue suspension. Homogenized tissue suspensions were introduced by pipette; in experiments with minced or chopped tissue this was weighed in the dry vessel before adding the solutions. The taps were closed 6 min. after immersing the vessels in the bath at 38° and readings were taken every 5 min. for 25 min. The rate of shaking was 132 oscillations/min. and it was found that O_2 uptakes of well over 1000 μl./hr. could be measured without uncertainty due to diffusion effects. In general, amounts (0·25–2 ml.) of tissue suspension were chosen such that the O_2 uptake was well below 1000 μl./hr. In the large majority of experiments the O_2 uptake/time curve was linear, at least after the first 5 min. The rate was taken from the linear part of the curve or in the few cases where the rate fell off appreciably the initial rate was accepted.

Succinoxidase

For the estimation of succinoxidase activity, equal amounts of the suspension were pipetted into both vessels of the Barcroft manometer and neutralized succinic acid solution was measured into the right-hand vessel. No alkali papers were put in the inner cups since no CO_2 is evolved in the oxidation of succinate to fumarate. Elliott & Schroeder [1934] showed that kidney slices oxidized fumarate rapidly, while with finely minced kidney practically no oxidation with fumarate took place though the succinic oxidation still occurred rapidly. Experiments with homogenized kidney, muscle, and other tissues, showed that no appreciable oxidation of fumarate occurred with the amounts of tissue used and at the concentration of fumarate which was likely to be present.[1]

In order to obtain a comparison of the succinoxidase activities of various tissues, it was first necessary to determine the conditions under which maximum activity would be observed in any tissue. While disintegration of tissue does not destroy succinoxidase, it is known [e.g. Lehmann, 1929] that violent shaking does inactivate it. With tissues having high activity, experiments showed that the activity observed after homogenization by Elvehjem's apparatus was greater than with minced or chopped tissue, probably owing to better dispersion. With several tissues having low activity, homogenizing did decrease the activity somewhat; it is possible that the violent breaking up of these tissues which were all rather tough has some destructive effect on the enzymes appreciable in comparison with the small activity of these tissues (see Table I).

Potter & Elvehjem [1937] carried out some measurements of succinoxidase activity, but the results given by them are considerably lower than those reported below (e.g. for rat kidney and liver suspension they found O_2 uptakes respectively of 5932 and 3895 μl./hr. per 1000 mg. tissue, against our figures of 22,600–33,200 and 13,300–24,000). Possibly their method of using only 1 ml. of fluid instead of 3 ml., in order to facilitate diffusion, was not quite satisfactory with the high concentration of tissue (40 mg.) which they used. They found that the maximum O_2 uptake occurred with 0·3 % succinic acid in the medium. In this work with all the tissues studied the optimum concentration was found to be about 0·9 %, and this concentration was in general used, though in most cases the O_2 uptake was measured at two or more different concentrations. The rate was often appreciably smaller with 0·6 % or less, or with 1·2 % succinic acid.

It is known that breaking up a tissue into a suspension lowers the respiration considerably compared with the respiration of thin slices. Elliott & Schroeder [1934] suggested that this is due to a dilution of coenzymes etc. and Krebs [1935] and Potter & Elvehjem [1936] showed that with a suspension the rate of respiration per unit weight increases with increasing concentration of tissue.[2] However, Potter and Elvehjem [1936] found that, unlike the residual respiration, the succinoxidase activity of tissues per unit weight is independent of the concentration of the tissue. We have confirmed this with all the tissues studied, by varying the amount of tissue in the 3 ml. of medium; also, in two experiments, the same rate of O_2 uptake was observed with equal small amounts of liver

[1] In later experiments it was found that fumarate increased the O_2 uptakes when higher concentrations of tissue were used. The extra O_2 uptake was largely balanced by extra CO_2 evolution.

[2] With the very dilute suspensions in phosphate buffer solution used in this work, the O_2 uptake in the absence of added substrate varied between 0 and 20 μl. in the 25 min. experimental period.

tissue in 3 ml. or in only 1 ml. of medium. Nevertheless, the succinoxidase activity was determined with two or more concentrations of tissue in nearly every case.

It was found that the activity of a liver, kidney, or heart tissue suspension sometimes, but not always, increased up to 35 % (70 % in one instance) during 1 or 2 hr. standing in the refrigerator. After this period the activity remained constant for a long time. Presumably slow solution of constituents of the tissue results in the better dispersion of some constituent of the enzyme system. In nearly every case the succinoxidase activity was determined at least twice,

Table I. *Succinoxidase activities of rat tissues*

		Succ Q_{O_2}	
Tissue	Preparation	On moist wt.	Mean of homogenized On dry wt.
Whole kidney	Homogenized	33·2, 30·4, 22·6, 18·9	112
Liver	Latapie minced	13·9	—
	Homogenized	20·2, 24·0, 13·3, 25·8, 21·8, 16·1, 13·8	66
Heart	Chopped	7·3	—
	Homogenized	18·4, 12·8, 12·9, 13·0, 12·1, 13·2	62
Whole brain	Ground to paste	3·9	—
	Homogenized	3·8, 4·2, 3·7, 3·2, 3·5, 4·0, 4·6, 4·3, 6·2	18
Testis	Ground to paste	1·6	—
	Homogenized	1·9, 1·5, 1·2, 1·7, 1·1, 1·9, 2·0, 1·7	13
Adrenal	Homogenized	3·6	11·7
Lung*	Chopped	0·6	—
	Homogenized	1·8, 1·8, 0·9	7·5
Skeletal muscle	Latapie minced	2·5, 3·1, 2·4	—
	Chopped	2·3	—
	Homogenized	1·5, 1·7, 1·4, 1·9, 1·3	6·6
Thymus	Chopped	0·51	—
	Homogenized	0·33	1·6
Pancreas	Chopped	0·24, 0·13	—
	Homogenized	0·0, 0·02	0
Spleen	Chopped	0·13	—
	Homogenized	0·16, 0·23, 0·04, 0·09	0·5
Blood†	Laked	0, 0	0
Whole foetus (1·1 g. each)	Chopped	0·73	—
	Homogenized	1·90	17·4
Jensen sarcoma (intramuscular)	Chopped	0·6‡	—
	Homogenized	2·1, 2·7, 2·1	13
Jensen sarcoma (subcutaneous)	Homogenized	2·0	12·9
Flexner Jobling carcinoma	Homogenized	2·0, 0·4	12·8, 2·1
Walker No. 256 carcinoma	Chopped	0·08	—
	Homogenized	0, 0·1, 0·1, 0·16, 1·0	0·6, 7·2
Philadelphia No. 1 sarcoma	Chopped	0·13	—
	Homogenized	0·11, 0·40, 0	1·0
Spontaneous mammary carcinomata	Chopped	0·21	—
	Homogenized	0·03, 0·18	0·5

* The O_2 uptake rates of rat and rabbit lungs with succinate fell off rapidly with time.

† The blood was taken from the hearts of etherized animals and run into water containing a trace of heparin. Tests showed that neither traces of ether nor of heparin affected succinoxidase.

‡ Tissue clumped together and rate fell off rapidly.

Table II. *Succinoxidase activities of rabbit and other tissues*

Tissue	Preparation	Succ Q_{O_2} On dry wt.
Rabbit kidney cortex	Homogenized	65·7
Rabbit kidney medulla	Homogenized	24·5
Rabbit liver	Latapie minced	34·5
	Homogenized	36·2
Rabbit heart	Homogenized	150
Rabbit testis	Homogenized	9·2
Rabbit lung	Chopped	6·1
	Homogenized	8·3
Rabbit skeletal muscle	Latapie minced	2·1
	Homogenized	1·5
Chick embryo (7 day)	Chopped	0·7
	Homogenized	2·0
Ox retina	Intact	10·3 } 3·9 }
	Homogenized	10·1 } 11·9 }

at intervals of 1 or 2 hr. When this was not convenient the determination was made 2 or 3 hr. after preparing the suspension.

To express succinoxidase activity the term Succ Q_{O_2} will be used where

$$\text{Succ } Q_{O_2} = \frac{\mu\text{l. } O_2 \text{ taken up in the oxidation of succinate}}{\text{hr.} \times \text{mg. tissue}}.$$

Tables I and II give the activities found in a number of tissues. The results are calculated on the moist weight and on the dry weight of tissue taken, the dry weight being deduced from the wet weight/dry weight ratio of the separately dried sample of tissue.

It will be seen in Tables I and II that the distribution of activity is very similar in the tissues of the rabbit and the rat. According to their succinoxidase activities the tissues fall roughly into three groups. (1) Kidney, liver and heart muscle have very high activities.[1] (2) Brain, testis, skeletal muscle, lung, adrenal, retina, Jensen sarcoma and Flexner-Jobling carcinoma, have fairly low activities. (3) Thymus, pancreas, spleen, blood, Philadelphia No. 1 sarcoma (usually), Walker No. 256 carcinoma, and two spontaneous mammary carcinomata (rat) have only a trace of activity or none at all. Battelli & Stern [1910] noted the high activities of kidney, liver and heart, but the activities observed with the crude methods available to them were several times lower than those now found. At present there does not seem to be any clear connexion between metabolic activity and succinoxidase activity. It is interesting to find tissues with normal respiratory activity but with practically no succinoxidase, as with spleen and several tumours. Absence of succinoxidase is not a definite characteristic of cancer tissue since some types of tumours, e.g. the Jensen sarcoma, always have definite activity. Fleisch [1924] and Boyland & Boyland [1936] have also found succinoxidase in the Jensen sarcoma. It does not seem to be a characteristic even of a single strain since with the Walker No. 256 one crop[2] was found to be quite active.

[1] The activity of these tissues is so high that very dilute suspensions of them are useful as quickly obtainable preparations of "succinoxidase" for use in rough estimations of succinate.

[2] For a set of experiments with small tumours, five or six young tumours implanted at the same time in several rats from one tumour were used.

The dehydrogenase

The succinoxidase activity as measured in the previous section is liable to be limited by the amount of any one of the components of the catalytic system. It was of interest, therefore, to obtain separate estimates of the specific succinate enzyme, i.e. the dehydrogenase, by an adaptation of the well-known methylene blue reduction technique of Thunberg. In the reduction of methylene blue no O_2 activator or carrier is concerned and only the rate of H transfer from succinate to methylene blue under the influence of the dehydrogenase is measured.

The Thunberg tubes used were of the Keilin [1929] type with a bulb in the stopper. The stopper bulbs contained 0·5 ml. 0·703 % methylene blue, this concentration being chosen so that 0·475 ml. contains methylene blue equivalent as H acceptor to 10 μl. O_2. The main tube contained a total of 2·5 ml. fluid consisting of tissue suspension in $M/10$ buffer, neutral succinate solution, water and enough extra buffer to make with the tissue suspension a total of 1 ml. phosphate buffer ($M/10$, pH 7·4). Control tubes contained the same additions with water instead of succinate solutions. Up to 10 tubes were evacuated simultaneously by means of a brass tube with 10 side tubes, connected to an oil suction pump and manometer. The tubes were evacuated three times and refilled with N_2 freed from O_2 by passage through a tube of hot copper wire fragments. They were finally evacuated, firmly closed, and the side tubes filled with water. In order to prevent the tissue from settling out, the tubes were fixed into a series of clips on a horizontal beam which was attached to the shaking apparatus of an ordinary Barcroft manometer tank; the tubes used were bent through an angle of about 40° and the clips were fixed at such an angle that the main parts of the tubes were nearly horizontal and the motion of the shaker kept the contents of the tubes well mixed. The main parts of the tubes were immersed in the bath water at 38°. The tubes were shaken in the bath for 1–3 min. before mixing the methylene blue at zero time. 1 min. was found to be sufficient to bring the fluid almost to the temperature of the bath. In open test tubes, mixtures were made of the same amounts of tissue suspension and buffer as in the Thunberg tubes, with water added to make a total of 3 ml., and 0·025 ml. of the methylene blue solution were added. These comparison tubes were frequently shaken with air to keep the methylene blue oxidized. The time of reduction was taken as the time when the colour in the Thunberg tube matched the colour in the comparison tube containing the same amount of tissue.

The activity of the succinic dehydrogenase will be expressed by the term Succ Q_{MB} where

$$\text{Succ } Q_{MB} = \frac{\mu\text{l. } O_2 \text{ equivalent of methylene blue reduced by the succinate system}}{\text{hr.} \times \text{mg. tissue}}$$
$$= M\frac{t - t_{Succ}}{t} \times \frac{60}{t_{Succ} \times w},$$

where $M = O_2$ equivalent of the total methylene blue reduced,

t = time in min. for reduction in the absence of succinate,

t_{Succ} = time in min. for reduction in the presence of succinate.

$\frac{t - t_{Succ}}{t}$ = fraction of total methylene blue reduced which is reduced by succinate,

w = weight of tissue in mg.

Table III. *Examples of the determination of succinic dehydrogenase activity of homogenized rat tissues*

Tissue	mg. moist	t	t_{Succ}	Succ Q_{MB}
Kidney	20	∞	8, 8, 8	3·8
	10	∞	15, 15	4·0
	5	∞	38, 38, 43	3·0
		6 hr. later		
	20	∞	8, 8, 8	3·8
	10	∞	13, 16, 16	4·0
	5	∞	32, 34, 34	3·7
Liver	50	109	6, 5·5	2·0
	25	360 ±	12, 12	1·9
	12·5	∞	23, 26	2·0
		3 hr. later		
	12·5	∞	27, 27	1·8
Heart	50	210 ±	3·5, 3·5	3·4*
	25	350 ±	6, 6	3·9
	12·5	∞	10·5, 10·5	4·6
	12·5	∞	12, 11·5	4·1
	6·25	∞	25, 26	3·8
	3·13	∞	67, 67	2·9†
Brain	100	35, 40	5·5, 5·5	0·95
	50	126, 141	12·5, 13	0·86
	25	600 ±	26, 26	0·88
Testis	200	23, 21·5	8·5, 8·5	0·22
	100	60, 61	20, 19	0·20
	50	425 ±	43, 39	0·26
Lung	200	27, 35	7, 7	0·37
	100	171 ±	34, 34	0·16†
	50	∞	83, 83	0·16†

Tissue	mg. moist	t	t_{Succ}	Succ Q_{MB}
Muscle	200	23, 28	8·5, 8·5, 8·5	0·24*
	100	77, 84	14·5, 14·5, 14·5	0·34
	50	200 ±	24, 21·5, 22	0·47
		4 hr. later		
	100	72, 75	14, 14·5	0·34
	50	200 ±	23, 23	0·46
	25	500 ±	51, 51, 49	0·46
Spleen	200	10·5, 10·5	7, 7	0·14*
	100	32, 32	12, 12	0·31
	50	155 ±	29, 28	0·34
Blood	240	∞	∞	0
	120	∞	∞	0
	60	∞	∞	0
Jensen sarcoma	133	50, 40	15, 15, 16	0·20
	70	120, 120	38, 31	0·18
	35	∞	105, 89	0·12†
Walker No. 256 carcinoma	195	40, 48	19·5, 14·5	0·11
	98	190 ±	49, 59	0·08
Phila. No. 1 sarcoma	200	7·5, 6·5	5·5, 5, 5·5	0·15*
	160	25, 29	8, 8	0·33
	100	86, 74	22·5, 22·5	0·19
	80	108 ±	46, 46	0·09†

Accurate determination of the reduction time with spleen is difficult due to the large amounts of haemoglobin present which is reduced in the vacuum tubes and oxygenated in the comparison tubes.

* The method often does not show the maximum activity when the reduction time is very short.

† With long reduction times inactivation of the enzyme seems to occur. With lung this is particularly marked. The succinoxidase activity of lung (rat and rabbit) also falls off rapidly in aerobic experiments.

Table IV. *Succinic dehydrogenase activities of homogenized rat tissues*

Tissue	Succ Q_{MB}* On moist wt.	Succ Q_{MB}* Mean on dry wt.
Kidney	3·9, 2·2	12·9
Liver	2·0, 1·3, 1·8, 1·4	5·1
Heart	3·4, 4·1	17·1
Brain	0·81, 0·88	3·9
Testis	0·16, 0·23	1·5
Lung†	0·32, 0·38	1·8
Muscle	0·42, 0·51, 0·59	2·1
Spleen	0·25, 0·31, 0·32	1·3
Blood	0	0
Jensen sarcoma	0·20, 0·22	1·3
Walker No. 256 carcinoma	0·06, 0·10	0·5
Phila. No. 1 sarcoma	0·22	1·3

* Widely variant results, see footnotes to Table VII, omitted.

† Uncertain, see footnote to Table III.

Under the conditions chosen for these experiments, $M=10$,

whence $$\text{Succ}\ Q_{MB}=\frac{600}{t_{Succ}\times w}\times\frac{t-t_{Succ}}{t},$$

or when the reduction by the tissue alone is very slow compared with the time in the presence of succinate, i.e. when t is much greater than t_{Succ},

$$\text{Succ}\ Q_{MB}=\frac{600}{t_{Succ}\times w}.$$

This formula is of course only an approximation, since it is assumed that the rate of reduction by substrates in the suspension is unchanged by the presence of succinate, and that the rate of reduction by succinate and other substrates is independent of the methylene blue concentration. Further uncertainty may be introduced by adsorption of the dye on tissue particles, and by progressive inactivation of both the succinic dehydrogenase and the other reducing mechanisms. However, the method serves to give a rough comparison of the dehydrogenase activities of the various tissues. While the time of reduction without succinate (t) tends to increase very rapidly with dilution of the tissue, the succinic dehydrogenase activity as measured by this method is usually approximately proportional to the amount of tissue. Occasionally rather wide variations in Succ Q_{MB} were found, especially with tissues of low activity, or when the reduction time was very short or very long. No appreciable change in activity was noticed in suspensions kept in the ice box for a few hours. Table III illustrates these points. The optimal concentration of succinate was found to be about 0·2–0·5 %; with very low concentrations the maximum rate is not reached, and higher concentrations cause definite inhibition. It will be noticed that the optimal concentration of succinate is lower for the reduction of methylene blue than for O_2. It is possible that excess succinate causes over-saturation of the enzyme surface preventing access of the dye, while with O_2 the reaction with the carrier and oxidase is so much more rapid that succinate is removed from the dehydrogenase surface rapidly enough to prevent oversaturation at this concentration.

For routine determinations, 10 mg. succinic acid in the 3 ml. (0·33 %) were used. Tubes were set up in duplicate or triplicate and two or more different amounts of tissue were used; usually amounts which would give reduction times 8–35 min. in the presence of succinate were chosen. In Table III examples of dehydrogenase determinations are shown and the results of determinations on a number of tissues are summarized in Table IV.

The value for Succ Q_{MB} is always much lower than Succ Q_{O_2} [see also Bach & Michlin, 1927; Wieland & Frage, 1929], which indicates that the reaction occurs less readily with methylene blue than with the natural carrier, cytochrome. The various tissues fall roughly into the same order according to dehydrogenase activities as they do according to their complete succinoxidase activities. However there is no constant relation between Succ Q_{MB} and Succ Q_{O_2}, a point which will be discussed further in a later section.

p-*Phenylenediamine oxidase*

Battelli & Stern [1912, 1] showed that all the tissues of higher animals have the power of oxidizing *p*-phenylenediamine and showed the variation of activity between different tissues. Several authors [Holmes, 1930; Penrose & Quastel, 1931; Quastel & Wheatley, 1932] have used the rate of oxidation of *p*-phenylenediamine as a measure of the "indophenol oxidase" or "respiratory ferment"

activities of various tissues. Quastel and his collaborators showed that the activity of the oxidase is not affected by destruction of the cell structure. In the next section it will be shown that since both the oxidase and cytochrome are concerned in the oxidation of *p*-phenylenediamine a true estimate of the full oxidase activity of tissues is not obtained unless excess cytochrome is present. However, the *p*-phenylenediamine oxidase, i.e. the oxidase plus cytochrome as present in the tissue suspensions, has been estimated in a number of tissues.

The procedure was similar to that for measuring succinoxidase activity. The *p*-phenylenediamine solution, freshly prepared each day, was added to both vessels of the manometer to compensate for the small autoxidation of the base (20–30 μl. per hr.). Tissue suspension was added only to the right-hand vessel. In a separate experiment the residual respiration of the tissue suspension at the concentration used was determined. This was always negligibly small and it was not deducted from the O_2 uptake observed with *p*-phenylenediamine since most of it probably takes place, like the *p*-phenylenediamine oxidation, through the mediation of the oxidase and cytochrome.

The rate of O_2 uptake increases rapidly with increasing *p*-phenylenediamine concentration up to about 0·6 %, and the optimum concentration was found to be about 1 %, further increase often producing a slight inhibition. In all estimations, therefore, the vessel fluid contained 30 mg. of the base in the 3 ml. In some cases the rate of *p*-phenylenediamine oxidation per unit weight of tissue increased somewhat with increasing concentration of tissue. Examples of this effect are shown in Table V. Duplicate determinations of O_2 uptake rates with *p*-phenylenediamine within a few hours often varied somewhat, but no constant effect of time of standing suspensions was noticed.

To express the rate of *p*-phenylenediamine oxidation, the term *p*-Phen Q_{O_2} will be used, where

$$p\text{-Phen } Q_{O_2} = \frac{\mu\text{l. } O_2 \text{ taken up in the oxidation of } p\text{-phenylenediamine}}{\text{hr.} \times \text{mg. tissue}}.$$

In Table VI the results obtained with various tissues are summarized. Again the order of the activity of the various tissues is about the same as for succinoxidase and the dehydrogenase. The activities found are several times higher than those given by Battelli & Stern [1912, 1] to whom only crude methods were available and who used tissues kept 5 hr. before mincing. As was noticed by Battelli & Stern, the rate of succinate oxidation by liver and kidney is somewhat higher than that of *p*-phenylenediamine (see Table VII). Both *p*-phenylenediamine and the succinate-dehydrogenase system react readily with cytochrome *c*, but it is possible that, with some tissues, structural arrangements on the tissue particles enable the latter system to react more rapidly than *p*-phenylenediamine with the insoluble cytochromes, *a* and *b*.

It is probable that both *p*-phenylenediamine and succinate-dehydrogenase can react with cytochromes *a* and *b* since in experiments in which the amount of soluble cytochrome was reduced by repeated washing of the tissue (brain, testis) the relative fall in O_2 uptake was small and about the same with either substrate.

The case of blood is exceptional. Whole rat blood diluted (and cytolysed) with water was used. With constant *p*-phenylenediamine concentration (30 mg. in 3 ml.), the O_2 uptake rate increased with increasing blood concentration up to about 200 mg. blood/3 ml.; further increase in blood concentration caused no further increase in O_2 uptake rate. The rate was steady, and it was quite low so that O_2 diffusion could not have been the limiting factor—increasing the surface by speeding up the shaking had no effect. It was noticed that the absorption

Table V. *Effects of tissue concentration and added cytochrome concentration on the* p-*phenylenediamine oxidation of rat tissues*

		p-Phen Q_{O_2} (on moist wt.)															
	Cytochrome added	mg. tissue in 3 ml.															
Tissue	mg.	3·1	5	6·3	10	12·5	20	25	37·5	40	50	75	100	150	200	300	400
Kidney	{ —	—	—	—	—	24·6	—	24·9	—	—	27·9	—	—	—	—	—	—
	1·6	—	—	—	—	68·5	—	68·8	—	—	—	—	—	—	—	—	—
	{ —	—	—	—	—	—	21·8	—	—	23·7	—	—	—	—	—	—	—
	1·6	—	74·4	—	64·6	—	54·2	—	—	—	—	—	—	—	—	—	—
	3·2	—	72·0	—	66·7	—	57·8	—	—	—	—	—	—	—	—	—	—
Liver	—	—	—	—	—	—	—	12·5	—	—	12·8	11·9	—	—	—	—	—
	{ —	—	—	—	—	—	—	—	10·9	—	—	10·8	—	—	—	—	—
	1·6	—	—	—	50·5	—	46·1	—	38·0	—	—	—	—	—	—	—	—
	3·2	—	—	—	51·2	—	46·8	—	40·6	—	—	—	—	—	—	—	—
Heart	—	—	—	—	—	15·4	—	16·4	—	19·1	—	—	—	—	—	—	—
	{ —	—	—	—	—	23·2	—	25·6	—	—	—	—	—	—	—	—	—
	1·6	—	—	112·0	—	95·0	—	—	—	—	—	—	—	—	—	—	—
	{ —	—	—	—	—	25·0	—	27·5	—	—	28·8	—	—	—	—	—	—
	1·5	97·8	—	94·9	—	91·8	—	—	—	—	—	—	—	—	—	—	—
	3·0	114·5	—	116·5	—	112·6	—	—	—	—	—	—	—	—	—	—	—
Brain	{ —	—	—	—	—	—	—	8·8	—	—	9·3	11·3	12·4	—	—	—	—
	0·7	—	—	—	—	—	—	15·0	—	—	14·6	—	—	—	—	—	—
	1·4	—	—	—	—	—	—	15·1	—	—	15·3	—	—	—	—	—	—
	{ —	—	—	—	—	—	—	—	6·9	—	—	6·8	—	—	—	—	—
	1·5	—	—	—	29·5	—	29·7	—	24·4	—	—	—	—	—	—	—	—
	3·0	—	—	—	29·5	—	28·7	—	25·2	—	—	—	—	—	—	—	—
Testis	{ —	—	—	—	—	—	—	—	—	—	—	—	2·9	—	3·2	—	—
	0·7	—	—	—	—	—	—	—	—	—	—	—	6·9	—	6·5	—	—
	1·4	—	—	—	—	—	—	—	—	—	—	—	8·3	—	—	—	—
	{ —	—	—	—	—	—	—	—	—	—	—	—	—	3·2	—	2·8	—
	1·5	—	—	—	—	—	—	—	12·7	—	—	11·1	—	9·2	—	—	—
	3·0	—	—	—	—	—	—	—	13·1	—	—	11·5	—	10·0	—	—	—
Lung	{ —	—	—	—	—	—	—	—	—	—	—	—	—	—	1·1	—	1·0
	1·0	—	—	—	—	—	—	—	—	—	—	—	—	—	5·7	—	—
	2·0	—	—	—	—	—	—	—	—	—	—	—	—	—	6·8	—	—
Skeletal muscle	{ —	—	—	—	—	—	—	—	—	—	—	2·8	—	—	3·5	—	—
	0·5	—	—	—	—	—	—	—	—	—	—	9·3	—	—	9·3	—	—
	1·0	—	—	—	—	—	—	—	—	—	—	10·1	—	—	10·1	—	—
Spleen	{ —	—	—	—	—	—	—	—	—	—	—	—	1·6	—	1·4	—	—
	0·7	—	—	—	—	—	—	—	—	—	—	—	5·6	—	—	—	—
	1·4	—	—	—	—	—	—	—	—	—	—	—	6·7	—	—	—	—
Jensen sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	3·3	—	3·9	—
	{ —	—	—	—	—	—	—	—	—	—	—	—	—	1·8	—	1·6	—
	1·5	—	—	—	—	—	—	—	8·1	—	—	6·6	—	5·2	—	—	—
	3·0	—	—	—	—	—	—	—	8·0	—	—	7·4	—	5·8	—	—	—
Phila. 1 sarcoma	{ —	—	—	—	—	—	—	—	—	—	—	—	—	—	0·8	—	0·8
	1·0	—	—	—	—	—	—	—	—	—	—	—	—	—	4·6	—	—
Blood	{ —	—	—	—	—	—	—	—	6·6	—	—	4·6	2·8	—	1·5	—	0·7
	1·0	—	—	—	—	—	—	—	6·9	—	—	—	—	—	1·5	—	—

Table VI. p-*Phenylenediamine oxidase activities of rat tissue suspensions*

	p-Phen Q_{O_2}	
Tissue	On moist wt.	Mean on dry wt.
Kidney	16·4, 17·7, 21·9, 20·0, 24·7, 23·7	91
Liver	11·9, 10·9, 10·4, 13·6, 14·1, 10·2, 11·9, 10·9	40
Heart	20·5, 19·1, 23·4, 16·4, 18·8, 23·5, 27·5	96
Brain	8·4, 8·1, 8·9, 10·7, 8·4, 9·8, 9·8, 9·3, 6·8	43
Testis	3·0, 2·8, 3·1, 3·5, 3·2, 3·4, 2·5, 2·5, 3·1	24
Lung	1·1, 1·2	5·8
Retina (Ox)	1·5	13

	p-Phen Q_{O_2}	
Tissue	On moist wt.	Mean on dry wt.
Skeletal muscle	2·9, 1·8	10
Spleen	1·5, 1·3	6·2
Pancreas	0·5, 0·3	1·0
Jensen sarcoma	2·2, 2·9, 3·3, 1·3, 1·7	14
Flexner-Jobling	1·1, 0·6	5·2
Walker No. 256	0·5, 0·3, 0·5, 0·4,	2·6
Phila. No. 1	0·8, 0·7	4·2
Blood (maximum observed)	6·6	124

bands of oxyhaemoglobin could be observed with the higher amounts of blood, they were faint at the limiting concentration, and completely absent in the range where O_2 uptake was proportional to blood concentration. In the latter solutions it appeared that the haemoglobin was completely destroyed; no methaemoglobin band in the red could be seen nor was reduced haemoglobin regenerated on the addition of hydrosulphite. Addition of cytochrome had no effect on the oxidation. When the concentration of blood was kept constant at a high value (450 mg./3 ml.), the O_2 uptake increased greatly with increasing *p*-phenylenediamine concentration tending to a maximum rate at a very high concentration of the base. It seems probable that the actual catalyst of the oxidation is a breakdown product of haemoglobin formed by a reaction between haemoglobin and *p*-phenylenediamine, and the amount formed is a function of both haemoglobin and *p*-phenylenediamine concentrations. Entirely similar results were obtained with crystalline horse haemoglobin; these results are shown in Figs. 1 and 2. The haemoglobin solutions were estimated by the ferricyanide method.

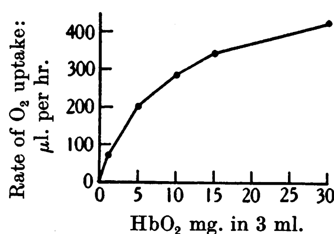

Fig. 1.

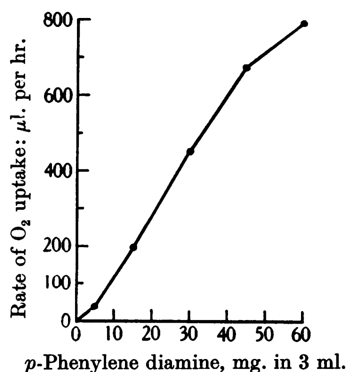

Fig. 2.

Fig. 1. Effect of varying HbO_2 concentration, with constant (30 mg.) *p*-phenylenediamine.

Fig. 2. Effect of varying *p*-phenylenediamine concentration, with constant (30 mg.) HbO_2.

Cytochrome

No attempt has been made to estimate cytochrome in the tissues directly, but some information about the quantitative relations of oxidase, cytochrome and dehydrogenase in tissue suspensions has been obtained by experiments in which known amounts of cytochrome were added.

Solutions of pure oxidized cytochrome *c* were obtained from ox heart muscle by the simple method of Keilin & Hartree [1937]. The cytochrome-iron content was estimated[1] by the αα-dipyridyl method [Hill & Keilin, 1933].

The experiments were carried out exactly as in the previous sections with 0·1–0·4 ml. of the cytochrome solution added to the medium in the vessels. O_2 uptake rates of various tissues with *p*-phenylenediamine and with succinate in the presence and absence of added cytochrome *c* are shown in Table VII.

Effect on p-*phenylenediamine oxidation.* Formerly it was believed that the oxidation of *p*-phenylenediamine, or the "Nadi" reagent, was catalysed directly by the oxidase, but it will be seen that the oxidation of *p*-phenylenediamine is greatly accelerated by the addition of cytochrome with all the homogenized

[1] These estimations were kindly made for us by Dr H. K. Alber.

Table VII. *Results of determinations, on the same sample of rat tissue, of the rates of oxidation of succinate and* p-*phenylenediamine, separately and together, with and without added cytochrome*

Homogenized suspensions used, unless otherwise stated. Activities calculated on moist weights.

Tissue	Cytochrome mg. in 3 ml.	Succ Q_{MB}	Succ Q_{MB} / Succ Q_{O_2}	Succ Q_{O_2}	*p*-Phen Q_{O_2}	*p*-Phen Succ Q_{O_2}
Kidney	—	—	—	22·6	16·4	24·0
	—	2·2	0·09	18·9	17·7	26·1
	2·0	—	—	17·4	36·4	34·9
Liver	—	—	—	13·3	10·9	13·1
	—	1·8	0·13	13·4	13·2	13·2
	0·7	—	—	13·2	19·1	12·6
	1·4	—	—	—	21·3	—
	—	1·4 ±	0·10	13·8	11·0	—
	0·5	—	—	14·4	23·0	14·1
Heart	—	—	—	13·0	23·4	26·0
	0·7	—	—	24·0	35·5	—
	1·4	—	—	23·8	42·2	43·3
	—	3·4	0·28	12·1	16·4	18·1
	1·4	—	—	17·8	43·4	28·6
Brain	—	0·81	0·20	4·1	8·4	14·3
	0·7	—	—	4·2	14·9	13·9
	—	—	—	4·0	8·9	14·4
	1·4	—	—	4·6	15·7	15·4
	3·5	—	—	—	15·8	—
Testis, Teased	—	—	—	0·94	4·3	3·1
Testis, Homogenized	—	—	—	1·2	2·8	3·8
Testis, Teased	—	—	—	0·69	5·0	3·5
	0·7	—	—	0·73	5·2	4·1
Testis, Homogenized	—	0·16	0·09	1·7	3·1	4·8
	0·7	—	—	1·7	6·7	4·8
	1·4	—	—	—	8·3	—
Lung	—	0·37	0·42	0·86	1·1	1·2
	2·0	—	—	1·7	6·8	6·0
Skeletal muscle	—	0·6	0·32	1·9	2·9	2·6
	1·0	—	—	2·8	10·1	5·7
Spleen	—	0·31	3·4	0·09	1·5	1·63
	0·7	—	—	0·85	5·6	2·3
	1·4	—	—	1·3	6·8	—
	—	—	—	0·04	—	—
	0·7	—	—	1·0	—	—
	1·4	—	—	1·7	—	—
Jensen sarcoma	—	0·22	—	2·0	2·9	3·6
	—	0·20	0·10	2·0	3·3	4·3
	1·0	—	—	1·9	4·7	4·8
Flexner-Jobling	—	—	—	2·0	1·1	1·3
	—	—	—	0·4	0·6	—
	1·6	—	—	1·8	5·0	—
Walker No. 256 carcinoma	—	—	—	0·16	0·51	0·4
	1·6	—	—	1·2	3·8	—
	—	0·06	0·7	0·09	0·27	0·37
	0·7	0·06	—	—	0·70	0·52
	1·4	—	—	0·20	0·96	—
Philadelphia No. 1 sarcoma	—	0·19	0·48	0·40	0·76	0·79
	1·0	—	—	1·09	4·6	3·3
	2·0	—	—	0·91	5·8	—
	—	—	—	0·0	0·65	—
	1·6	—	—	0·8	4·0	—
Pancreas*	—	—	—	0·04	0·49	—
	1·6	—	—	0·05	0·48	—

* Results with pancreas are doubtful. It is possible that enzymes are destroyed in suspensions of this tissue. Also the suspensions become acid fairly rapidly.

tissues so far tried; Ogston & Green [1935] found the same with an enzyme preparation from heart. The reduction of oxidized cytochrome *c* by a solution of *p*-phenylenediamine occurs immediately and can be readily observed with a hand spectroscope. The Nadi reagent also reduces cytochrome, and with a tissue suspension in an open test tube with added Nadi reagent the development of the blue colour throughout the solution occurs much more rapidly if a little oxidized cytochrome is added. It seems definite, therefore, that these oxidations take place through the mediation of cytochrome, and the oxidation which occurs without added carrier may be due to the cytochrome already present in the tissue suspension. (Keilin & Hartree [1938] have recently demonstrated the same points very thoroughly.)

From Table V it will be noticed that with added cytochrome the rate of *p*-phenylenediamine oxidation per unit weight of tissue increases toward a maximum with decreasing concentration of tissue and increasing concentration of cytochrome: i.e. the maximum oxidase activity is shown when the ratio of cytochrome concentration to tissue concentration is high. Keilin [1930] showed similar effects of cytochrome and enzyme concentration in the oxidation of cysteine by preparations of heart muscle indophenol oxidase.

It follows that to obtain a maximum estimate of the oxidase content of a tissue it is necessary to add excess cytochrome with the *p*-phenylenediamine, using low concentrations of tissue. In this way the oxidase activity of rat heart, for instance, is found to be equivalent to the remarkably high figure of $Q_{O_2}=500$ approximately (calculated on the dry weight of tissue). Even these high figures possibly do not represent the absolute maximum for the tissues, since the process of homogenizing probably does not result in the rupture of all cells and the extra cytochrome would not penetrate into intact cells. But it is seen that with every tissue the oxidase activity is considerably higher than is necessary to account for the whole normal respiration as measured by the tissue slice technique. In Table VIII estimates of approximately the full oxidase activities of a few tissues are given.

Table VIII. *Estimates of the full oxidase activities of rat tissues. Rates of O_2 uptake of tissue suspensions in the presence of* p-*phenylenediamine and excess of cytochrome* c

Tissue	*p*-Phen Q_{O_2} On moist wt.	*p*-Phen Q_{O_2} Mean on dry wt.	Tissue	*p*-Phen Q_{O_2} On moist wt.	*p*-Phen Q_{O_2} Mean on dry wt.
Kidney	68·8, 72·0	288	Spleen	6·8, 7·8	32
Liver	51·2	167	Muscle	10·1, 8·1	38
Heart	114·5, 112·0	506	Jensen sarcoma	8·0, 5·7	43
Brain	29·5	134	Flexner-Jobling carcinoma	5·0	28
Testis	13·1	106	Walker No. 256 carcinoma	1·0, 3·8, 1·3	15
Lung	6·8, 7·8	31	Phila. No. 1 sarcoma	4·0, 5·8	29
			Ox retina	10·6	88

With tissue in which the cells have not been extensively broken up by homogenization the results are somewhat different. This is seen (Table VII) in experiments with rat testis which was merely pulled apart into loose bunches of tubules. With this "teased" testis the *p*-phen Q_{O_2} was higher than with the homogenized tissue but addition of cytochrome scarcely affected the rate. Apparently the oxidation of the *p*-phenylenediamine takes place inside the cell

where there is a certain concentration of cytochrome; added cytochrome, not being permeable into the cells does not affect the rate. The homogenized tissue has a lower original *p*-phen Q_{O_2} due, perhaps, to the dilution of the cytochrome from the broken cells, but added cytochrome can now come into contact with the oxidase and so can raise the O_2 uptake rate even above that found in the intact cells with their limited content of carrier.

Effect of succinate oxidation. Results of experiments on the O_2 uptake of tissue suspensions in the presence of succinate with and without the addition of cytochrome are shown in Table VII.

With spleen only a trace of succinoxidase activity was found directly, although the experiments on methylene blue reduction showed the presence of dehydrogenase. It was therefore supposed that the lack of O_2 uptake with succinate was due to lack of carrier, and, as expected, it was found that on the addition of cytochrome the O_2 uptake was increased 10–40 times. With heart there is also a considerable acceleration (50–80 %) of succinate oxidation on adding cytochrome, which shows that, while this tissue contains cytochrome, the amount in a suspension is not enough to carry the rapid H transfer which the high dehydrogenase activity (Succ $Q_{MB} = 3{\cdot}1, 4{\cdot}1$) of the tissue is capable of activating.

With brain, testis, kidney and liver the addition of cytochrome does not appreciably affect the succinate oxidation, there being evidently enough cytochrome present to effect H transfer as fast as the dehydrogenase present can "activate" it. It will be noticed that the rate of methylene blue reduction by suspensions of these three tissues is particularly low compared with the rate of O_2 uptake, i.e. the ratio Succ Q_{MB}/Succ Q_{O_2} is particularly low. In any tissue in which this ratio exceeds about 0·2, it is found that the dehydrogenase activity exceeds the capacity of the cytochrome present to transfer all the H activated; the limiting factor is the concentration of cytochrome (oxidase always being present in excess) and accelerated O_2 uptake is obtained by adding cytochrome. It should be mentioned that experiments showed that the addition of cytochrome had no effect on the time of reduction of methylene blue; no carrier is required when methylene blue replaces O_2 and oxidase.

The rate of O_2 uptake with p*-phenylenediamine plus succinate*

With a number of tissues the O_2 uptake rate was determined in the presence of optimal concentrations of both succinate and *p*-phenylenediamine. The term ${}^{p\text{-Phen}}_{\text{Succ}}Q_{O_2}$ is used to express these rates. Results are given in Table VII. It will be seen that with the majority of homogenized tissues, in the absence of added cytochrome, *p*-phenylenediamine and succinate together gave an O_2 uptake greater than that obtained with either substrate separately. In fact with several tissues, notably brain and testis, the effect was equal to or greater than the sum of the effects of either substrate separately. With other tissues, notably liver, the "additive" effect is not apparent. Battelli & Stern [1912, 1, 2] noticed this "additive" effect with brain and its absence in liver, and considered it probable that the mechanisms for the oxidation of *p*-phenylenediamine and of succinate were not identical in brain. However, as can be seen in Table VII, the "additive" effect is not confined to brain; it occurs in most tissues, varying from an effect in excess of simple addition of the O_2 uptakes of the two substrates separately, in some experiments with brain, to complete absence of summation with liver. In view of the well-known effects of inhibitors such as HCN, it seems most probable that the oxidations of both *p*-phenylenediamine and succinate take place almost exclusively through cytochrome and indophenol oxidase. Many

experiments were tried to explain the "additive" effect until it was found that the oxidations of *p*-phenylenediamine by brain and testis could be accelerated not only by adding succinate but also by fumarate, malonate or acetate, none of which substances was itself rapidly oxidized by the suspensions in the absence of *p*-phenylenediamine. With liver, fumarate and malonate had little effect on the oxidation of *p*-phenylenediamine. (Acetate increased the O_2 uptake of liver with *p*-phenylenediamine markedly. This result was repeatedly observed but cannot yet be explained.) Some results are shown in Table IX.

Table IX. *Effects of Na salts of acids on* p-*phenylenediamine oxidation*

Salt added equivalent to 0·15 *M* Na in each case.

	Cyto-chrome	*p*-Phen Q_{O_2} on moist wt.				
		No addition	Succinate	Fumarate	Malonate	Acetate
Brain	−	9·8	16·6	14·7	15·3	14·4
	+	23·2	18·0	15·6	16·1	17·4
Testis	−	2·5	3·6	3·2	3·5	3·2
	+	7·4	4·0	3·3	3·4	3·8
Liver	−	11·9	15·0 (16·8)*	12·4	13·9	18·5
	+	40·0	23·9	18·3	19·3	30·9

* Succinate without *p*-phenylenediamine, i.e. Succ Q_{O_2}.

It seems most likely that the "additive" effect is in reality simply a salt effect of the Na salts of the acids added.

That succinate oxidation is not concerned in the observed stimulation of O_2 uptake of brain and testis with *p*-phenylenediamine, is shown by the following experiments. (1) Estimations of succinate indicated that, in the presence of *p*-phenylenediamine, succinate oxidation was inhibited. (Succinate was determined by the manometric succinoxidase method after removing the diamine with permanganate and extraction of the succinate with ether.) (2) Labes & Krebs [1935] and Potter & Elvehjem [1937] showed that selenite in low concentration inhibits succinic dehydrogenase and we have confirmed this both on O_2 uptake and methylene blue reduction. Selenite causes a small, 4–20 %, acceleration of *p*-phenylenediamine oxidation, and the high O_2 uptake of *p*-phenylenediamine plus succinate is also slightly further *increased* by selenite. If oxidation of succinate caused the additional O_2 uptake with *p*-phenylenediamine plus succinate, selenite would *reduce* the total O_2 uptake. Similar results were obtained with malonate which also is known to inhibit succinic dehydrogenase [Quastel & Wooldridge, 1928; Quastel & Wheatley, 1931]. (3) Finally, the formation of dark oxidation products of *p*-phenylenediamine with brain suspensions is appreciably more rapid when salts of succinate and other acids are added, as can be readily observed in test tube experiments. With liver these salts seem to delay the darkening. The inhibitory effect of the salts on the O_2 uptake with *p*-phenylenediamine plus cytochrome, mentioned below, can also be observed.

Battelli & Stern [1912, 2] noted that NaCl solutions up to 0·1–0·17 *M* increased the rate of *p*-phenylenediamine oxidation by brain mince. The succinate (presumably $C_4H_4O_4Na_2$, $6H_2O$) in the concentration used by them [1912, 1] corresponded to 0·12 *M* Na and so should be expected to accelerate *p*-phenylenediamine oxidation purely as a salt effect; and in fact it did so to just about the same relative extent (31 %, 35 %) as they found with NaCl in their next paper [1912, 2]. With liver mince, weak NaCl solutions, 0·05 *M* increased the rate of oxidation of *p*-phenylenediamine but with stronger solutions, $>0·1\,M$, the rate decreased again. With the amount of sodium succinate used by them it was to be expected that only a small increase in rate of O_2 uptake would be observed.

The mechanism of this salt effect is not clear. It will be noticed (Tables VII and IX) that with all the tissues the O_2 uptake rate with *p*-phenylenediamine plus excess cytochrome is usually lowered on the addition of succinate or other

salts. The salts may improve the adsorption of cytochrome on the oxidase, in brain and testis particularly, and so increase its rate of oxidation, thus increasing the O_2 uptake rate when cytochrome is the limiting factor; but when excess of cytochrome is present the activity of the oxidase is the limiting factor and salts may have a depressing effect on this. The difference between brain and liver, at the two extremes, may be due to differences in the colloidal behaviour of the tissue particles with which the oxidase is associated. Further work on these points is in progress.

Note. Breusch [1937], in a paper which appeared after the completion of this work, reports a similar distribution of succinoxidase activity, the activity being measured by the formation of fumarate $\rightleftharpoons$ malate. He did not observe the excess of cytochrome oxidase in the tissues.

Summary

1. Using tissues reduced to fine suspensions by Potter & Elvehjem's [1936] method, conditions have been worked out under which the approximate maximum rates of oxidation of succinate and *p*-phenylenediamine can be observed. A method of applying the Thunberg methylene blue reduction technique for the determination of succinic dehydrogenase in tissue suspensions is described. Using these methods estimates have been obtained of the activity of the complete succinoxidase system, the indophenol oxidase and the succinic dehydrogenase in various tissues.

2. The complete system, "succinoxidase", is extremely active in kidney, liver and heart. Brain, testis, skeletal muscle, lung, adrenals and retina have moderate to low activities. Thymus, pancreas, spleen and blood, have practically no activity. Cancer tissues vary; Philadelphia No. 1 sarcoma, Walker 256 carcinoma (usually) and certain spontaneous mammary carcinomata (rat) are inactive, while Jensen sarcoma, Flexner-Jobling and one example of Walker 256 carcinoma showed definite, though low, activities.

3. The oxidation of succinate by methylene blue is much slower than by O_2, and there is no constant relation in different tissues between the rate of reduction of methylene blue and the rate of O_2 uptake. But in general the tissues fall into the same order when arranged according to their activity measured in either way.

4. The tissues fall into roughly the same order according to their activity in oxidizing *p*-phenylenediamine.

5. The oxidation of *p*-phenylenediamine is effected by the indophenol oxidase through cytochrome and it is only in the presence of excess cytochrome that an estimate of the full oxidase activity of the tissues can be obtained. On adding excess of cytochrome *c*, the oxidation of *p*-phenylenediamine is considerably increased in all homogenized tissues. In this way it is found that the activity of the tissues is in all cases considerably higher than is necessary to account for the whole respiration of the normal tissue.

Since cytochrome does not penetrate cells, the effect of adding cytochrome is not apparent in incompletely disintegrated tissue.

6. Cytochrome is a necessary link in the complete succinoxidase system. In some tissues, the ratio between rate of methylene blue reduction (i.e. the dehydrogenase estimate) and rate of O_2 uptake is comparatively high. In these cases the O_2 uptake rate with succinate can be increased to a maximum value by adding cytochrome *c*.

7. In the presence of optimal concentrations of both *p*-phenylenediamine and succinate, many tissues, notably brain and testis, give O_2 uptake rates

greater than with either substrate separately. This effect seems to be due, not to a difference in oxidases concerned with the two substrates, but to a salt effect of the Na succinate on the oxidase-cytochrome activity; similar results were obtained with fumarate, malonate, and acetate. With liver the "additive" effect is absent. With all the tissues the high rate of *p*-phenylenediamine oxidation in the presence of added cytochrome is lowered on the addition of succinate and other salts.

8. Blood catalyses the oxidation of *p*-phenylenediamine strongly but the mechanism is quite different from that in other tissues. The catalyst concerned seems to be a breakdown product of haemoglobin produced by reaction with the diamine. Cytochrome addition has no effect.

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