CCXXIV. THE EFFECT OF CERTAIN BACTERIAL TOXINS UPON SOME RESPIRATORY MECHANISMS OF ANIMAL TISSUES

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LITTLE work has hitherto been done to ascertain the effect of bacterial toxins upon physiologically active enzyme systems. Walthard [1934] reported that the addition of diphtheria toxin in bouillon to a rat liver preparation in a Barcroft micro-respirometer exerted no action upon the rate of O_2 absorption. On the other hand, Cross & Holmes [1937] reported that the power of isolated liver tissue of rabbits to form extra carbohydrate in the presence of various substrates is much lower for tissue obtained from animals suffering from diphtheritic toxæmia than for tissue obtained from normal rabbits. They also showed that in the presence of pyruvate the rate of O_2 uptake of liver slices obtained from a toxæmic rabbit is subnormal.

In this paper experiments are reported in which various toxins obtained from members of the *Clostridium* group of bacteria have been added to suspensions of actively respiring tissues in a Barcroft differential micro-respirometer.

EXPERIMENTAL

Corbet & Wooldridge [1936] pointed out the necessity for replication¹ and repetition of experiments made with the Barcroft differential manometer, especially when comparisons are made between results which do not differ widely. To record a result merely as the average of so many experiments without giving the standard error makes the acceptance of such results difficult. Our own experience with tissue respiration studies reinforces us in our view that results are often misleading unless experiments be both replicated and repeated and the final results expressed statistically.

Most of the work described has been done with suspensions in tap water of small intestine minced in a Latapie mincer after washing to remove intestinal contents. Other tissues have been used and the technique of their preparation is similar, the tissues being minced in the Latapie apparatus (liver, kidney, skeletal muscle) or ground in a mortar (brain, heart muscle), suspended in tap water and filtered through muslin in order to obtain a more homogeneous suspension. Owing to the fact that the minced tissues actively oxidized succinate, and to the advisability for replication of results, which was relatively easy with suspensions of finely minced tissue, the tissue slice technique was not followed in this work.

The tissues were obtained from guinea-pigs, and were used within 1 hr. of the death of the animal. The small intestine was employed to a great extent as

¹ By "replication" is meant the setting up of a series of identical experiments at one and the same time.

Cl. welchii is frequently the cause of intestinal trouble in certain animals. In the manometric experiments tissue was placed in each cup, and usually the only difference between the contents of the left and right cups was the addition of substrate to the latter. Many substrates have been tried but mainly succinate has been used. The results given are based on experiments lasting 40 min. at 37°, in which the rate of O₂ absorption was still proceeding linearly. There was usually a diminution in the rate after 1 hr. The experiments were continued for 2 hr. to make sure that their course was normal. The majority of our experiments were put up in quadruplicate or higher replication and each experiment was usually repeated at least 5 times. In the tables the results are expressed as means, with the standard errors given in brackets. In order to facilitate statistical analysis, the mean of the replicated results from any particular experiment was expressed as 100 for the O₂ absorption in 40 min. by a substrate alone in the presence of a particular tissue but in the absence of toxin or antiserum. (The actual results varied in the different experiments between 80 μ l. and 150 μ l. for succinate.) The figures for the O₂ uptake in the presence of toxin or antiserum were altered in the same proportion to facilitate comparison.

The micro-respirometers contained air and were shaken at 37°, the readings being started after 10 min. temperature equilibration. A roll of filter paper soaked in KOH solution, to absorb CO₂, was present in the centre cup of all manometric flasks.

The toxins and antitoxins used were obtained through the kindness and generosity of Dr O'Brien, Prof. Dalling and Dr Montgomerie of the Wellcome Physiological Research Laboratories, Langley Court, Beckenham, Kent, England. These toxins were prepared from broth filtrates of cultures of the appropriate anaerobe by precipitation with 70% ammonium sulphate, the deposit being dried *in vacuo*. The broth used was made from horse muscle with the addition of Parke Davis peptone and a large proportion of cooked meat in the case of *Cl. welchii*, types A and D, and of Witte peptone with only a small amount of cooked meat in the case of the type C organism. As a control for these toxins a similar preparation was made from the uninoculated broth used for *Cl. welchii*, type A, and this is alluded to in the tables and the text as the broth precipitate or broth precipitate control. The strength of toxin usually used in the manometric flasks was 0.4 mg. dried material per flask. The tissues used were usually washed and suspended in tap water but the contents of the flasks were maintained at pH 7.4 by the addition of 0.5 ml. *M*/20 phosphate buffer. No change in pH occurred during the experiments. The average dry wt. of tissue (small intestine) added to the flasks was 14 mg., but it varied between 11 and 16 mg. and on occasion was as high as 20 mg. Where toxin and antiserum were used in the same cup they were mixed and allowed to stand for 15 min. before adding to the tissue.

Aerobic oxidations by minced small intestine. Krebs [1933; 1935] and others have reported no marked oxidative activity of small intestine with various substrates. Using aqueous suspensions of minced small intestine in the micro-respirometer we have been unable to show any definite oxidizing activity for any substrate other than succinate, *p*-phenylenediamine and dihydroxyphenylalanine (Table I). Under our experimental conditions we were unable to show any activity with glucose, fructose, sucrose, lactate, pyruvate, fumarate, malate, oxalate, butyrate, oxaloacetate, citrate, formate, glycerol, alanine, serine, tryptophan, glutamate, aspartate, phenylalanine, glycylglycine, leucylglycine, glycyltryptophan or glycyltyrosine.

Table I. *Aerobic oxidations by small intestine*

Experimental details in the text. Each experiment with succinate and with *p*-phenylenediamine was quadruplicated and with dihydroxyphenylalanine was triplicated. The *p*-phenylenediamine was used as the hydrochloride neutralized with NaOH. The mean values are given for the dry wt. of tissue used and for the " Q_{O_2} ". The O_2 absorbed is due to the substrate in the presence of the tissue, the tissue being present in both cups, hence " Q_{O_2} " here represents the difference in true Q_{O_2} caused by substrate addition.

Substrate	No. of exps.	Dry wt. of tissue	Q_{O_2}
Succinate <i>M</i> /120	30	13.70	11.73 (S.E. = 0.47)
<i>p</i> -Phenylenediamine <i>M</i> /54	12	15.85	10.33 (S.E. = 0.20)
Dihydroxyphenylalanine <i>M</i> /120	5	16.78	3.65 (S.E. = 0.40)

The effects of various bacterial toxins upon the aerobic oxidation of succinate in the presence of small intestine. Various toxins obtained from certain anaerobic bacteria were added to mixtures of succinate and small intestine in Barcroft micro-respirometers, and their effects upon the rate of aerobic oxidation observed. It will be seen from Table II that all the toxins inhibit the oxidation to some small

Table II. *The effects of various bacterial toxins upon the aerobic oxidation of succinate in presence of small intestine*

The figures given for the O_2 uptake represent the means, and their standard errors, obtained from the numbers of experiments mentioned. Details in text. The meaning of the symbols used is as follows. *S* = *M*/120 succinate, BP = control broth precipitate, A, C and D represent the toxins obtained respectively from *Cl. welchii*, types A, C and D. Oed., Sept., and Tet. represent the toxins obtained respectively from *Cl. oedematiens*, *Cl. septicus* and *Cl. tetani*. All toxins were used in a concentration of 0.4 mg. dried product per manometric cup. S.E. = standard error, and *P* might be called the probability of significance. If $P^* < 0.01$ this is regarded as significant.

	S	S + BP	S + A	S + C	S + D	S + Oed.	S + Sept.	S + Tet.
No. of exps.	121	9	63	39	18	6	6	5
No. of manometers	467	27	237	141	69	23	24	18
Mean O_2 uptake	100.11	82.41	15.79	35.91	70.52	77.80	86.53	81.63
S.E.	0.46	1.04	0.35	0.71	1.56	1.69	2.35	2.15

Values for *P* compared with:

(a) Succinate control	—	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
(b) Broth ppt. control	—	—	<0.01	<0.01	<0.01	0.1-0.2	0.2	0.8-0.9

* For methods of statistical calculations see Fisher [1936].

extent but that a similar inhibition is induced by the "broth precipitate". Only the toxins of *Cl. welchii* produce inhibitions significantly greater than that of the broth precipitate, and of these inhibitions that induced by the type A organism is greatest and that by the type D least. The extent of the inhibition varies with the amount of toxin added, as is shown in Table III, but at the concentration chiefly employed in these particular studies, viz. 0.4 mg. toxin per cup, the inhibition by the toxins of the A, C and D types of organism are respectively 84.21, 64.09 and 29.48 % (Table II). The proportion of the α , β and ϵ toxins present in these three toxins is given in Table III and it will be seen that the α toxin is present in all. The β toxin is only present in the toxin from the type C organism and the ϵ toxin only in that from type D, and, in comparison with α toxin, their concentrations are high. These results suggest, therefore, that the inhibition of the succinate oxidation may be due to the α toxin, but that, in any case the inhibitory actions of the β and ϵ toxins must be small.

Table III. *The effect of the toxins of Cl. welchii in different concentrations upon the aerobic oxidation of succinate by small intestine*

The upper part of the table indicates the nature of the toxin preparations used, and the lower half gives the % inhibition produced by the various toxins in the concentration indicated in the first column. Experiments were made in quadruplicate and the standard errors were small.

Toxin	Type A	Type C	Type D	Broth ppt. control
Units toxin per 100 mg.				
α	12.5	Some	Some	0
β	0	100	0	0
ϵ	0	0	800	0
Inhibition of succinate oxidation (%)				
Mg. toxin per cup				
0.01	68	49	—	—
0.40	83	65	30	19
1.6	98	93	60	38
2.4	—	—	74	39

The effects of antisera upon the inhibition induced by the toxins of Cl. welchii. The oxidation of succinate by small intestine is inhibited by the presence of the toxins of *Cl. welchii*, but if various antisera are added as well as the toxin the inhibition induced by the toxin is considerably diminished by those antisera which contain antibodies for the α toxin. Table IV gives the results obtained

Table IV. *The effects of various antisera upon the inhibition by the toxin of Cl. welchii, type A, of the aerobic oxidation of succinate in the presence of small intestine*

The figures given in the table are the means, and standard errors, of five experiments each made in quadruplicate. The results have been adjusted so that the mean for the O_2 uptake due to succinate alone is 100. The α toxin present in the toxin used (0.4 mg. per cup) could be neutralized by a 1/10,000 concentration of antiserum or a 1/2800 concentration of the type D antiserum, as judged by mouse-inoculation experiments. S=succinate, AS=antiserum, N=normal horse serum, TX=toxin; other abbreviations as for Table II.

Antiserum	S	S + AS	S + TX	S + TX + AS	% inhibition			% inactivation of TX
					TX	AS	(TX + AS) - AS	
<i>Welchii</i> A, 1/10,000	100	88.70	15.00	47.60	85	11.3	41.10	51.7
S.E.	2.26	2.50	1.21	1.43				
<i>Welchii</i> C, 1/10,000	100.37	96.50	18.05	16.48	81.95	3.87	80.02	2.4
S.E.	2.23	2.28	1.71	1.35				
<i>Welchii</i> C, 1/4000	100.01	84.56	14.10	37.08	85.91	15.45	47.48	44.7
S.E.	1.37	1.61	0.80	1.41				
<i>Welchii</i> D, 1/10,000	100.65	92.95	17.85	16.20	82.80	7.70	76.75	7.3
S.E.	2.14	3.04	1.54	0.93				
<i>Welchii</i> D, 1/2800	100.25	85.22	13.55	49.92	86.70	15.03	35.30	59.3
S.E.	2.87	2.37	0.70	1.70				
Normal, 1/2800	100	91.90	11.20	8.40	88.80	8.10	83.50	6.0
S.E.	2.25	2.14	1.19	1.16				
<i>Septique</i> , 1/2800	100.25	88.55	13.65	9.40	86.60	11.70	79.15	8.6
S.E.	2.19	3.28	1.63	1.63				
<i>Oedematiens</i> , 1/2800	99.75	84.25	14.70	9.33	85.05	15.50	74.92	11.9
S.E.	1.84	2.44	1.09	1.10				
<i>Tetani</i> , 1/2800	100.27	85.10	13.85	8.35	86.42	15.15	76.75	11.2
S.E.	1.59	1.97	1.11	1.12				

with the toxin of *Cl. welchii* type A and various antisera, these sera being added in amounts equal to, or greater than, the concentration of the homologous antiserum which should neutralize the toxin, viz. 1/10,000. Of the antisera used only those for the three types of *Cl. welchii* contained antibodies for the α toxin. The antitoxic strengths of these three antisera were as follows: type A antiserum contained 300 units of α antitoxin per ml., type C antiserum contained 120 units of α antitoxin and 900 units of β antitoxin per ml. and type D antiserum contained 85 units of the α antitoxin and 275 units of the ϵ antitoxin per ml. From the results given in Table IV it is seen that when these three antitoxins are added in amounts equivalent in α antitoxin units they each reduce the inhibitory effect of the type A toxin by about half. The other antisera used and the normal serum produced no appreciable inactivation of the toxin. It will be noticed that all the sera themselves produced some small but significant inhibition of the succinate oxidation and this has been allowed for in calculating the extent of the

Table V. *The inactivation of the toxin of Cl. welchii, type A, by its homologous antiserum, as measured by the inhibition produced of the aerobic oxidation of succinate by small intestine*

Experimental details in the text. The figures given were obtained from replicated experiments and represent the O_2 absorbed.

Conc. of antitoxin	0	$\frac{1}{4} \times 10^{-4}$	$\frac{1}{2} \times 10^{-4}$	1×10^{-4}	2×10^{-4}	4×10^{-4}
No toxin	100	90	86	89	89	84
Toxin present	13	10	30	48	59	74

Table VI. *The effects of various antisera upon the inhibition by the toxins of Cl. welchii, type C and type D, of the aerobic oxidation of succinate in the presence of small intestine*

The figures given in the table are the means and standard errors of five experiments in the case of toxin C, and of fewer experiments in the case of toxin D, each made in quadruplicate. The results have been adjusted so that the mean for the O_2 uptake due to succinate alone is 100. The β toxin present in toxin C (0.4 mg. per cup) could be neutralized by a 1/3080 concentration of antiserum to the type C organism, while the ϵ toxin present in toxin D (1.6 mg. per cup) could be neutralized by a 1/66 concentration of antiserum to D (i.e. twice the actual concentration used), as judged by mouse inoculation experiments. S=succinate, AS=antiserum, Tx=toxin, N=normal horse serum and other abbreviations as for Table II.

					% inhibition			% inactivation of toxin
Toxin + AS	S	S + AS	S + Tx	S + Tx + AS	Tx	AS	(Tx + AS) - AS	
Toxin C:								
<i>Welchii</i> A, 1/3080	100.20	81.95	30.92	48.35	69.28	18.25	33.60	51.5
S.E.	2.12	3.12	2.60	2.57				
<i>Welchii</i> C, 1/3080	100.11	85.46	37.99	51.26	62.12	14.65	34.20	43.95
S.E.	1.87	1.54	1.21	1.55				
<i>Welchii</i> D, 1/3080	100.32	82.58	30.42	39.01	69.89	17.73	43.58	37.7
S.E.	1.60	1.58	1.93	1.80				
Normal, 1/3080	99.82	86.93	29.42	25.20	70.40	12.89	61.73	12.3
S.E.	2.12	2.20	1.92	0.96				
<i>Septique</i> , 1/3080	100.48	87.40	36.10	24.10	64.38	13.08	63.30	1.7
S.E.	2.22	2.91	1.09	1.43				
Toxin D:								
<i>Welchii</i> D, 1/134	100.33	90.2	58.66	70.5	41.67	10.13	19.70	52.7
S.E.	4.37	3.26	4.18	3.48				
<i>Welchii</i> A, 1/134	100.33	96.4	58.66	80	41.67	3.93	16.4	60.6
S.E.	4.37	4.31	4.18	3.29				

diminution of the inhibitory effect of the toxin on addition of serum. By varying the concentration of the antitoxin added the extent of the diminished inhibition is changed accordingly, as is shown in Table V. Results similar to those reported in Table IV for the toxin of *Cl. welchii*, type A, were obtained with the toxins of the organism, types C and D (Table VI). This table shows that the extent of the reduction of the inhibition due to these toxins is greatest with the antiserum for the type A organism, i.e. it is greatest with that antiserum containing the most α antitoxin. These results support the view that the inhibition of the succinate oxidation produced by the toxins of *Cl. welchii* is due to α toxin.

The effects of heated toxins upon the aerobic oxidation of succinate by small intestine. All the toxins used in this work are heat-labile, except that for the type D organism, the ϵ toxin in which is relatively heat-stable. If the inhibition of the succinate oxidation is due to any of these toxins except ϵ toxin, then it should be removed if the toxins are first heated to 80° for 15 min. The results given in Table VII indicate clearly that the small inhibitions induced by the

Table VII. *Comparison of the effects of toxins, before and after heating for 15 min. at 80°, upon the aerobic oxidation of succinate by small intestine*

The figures, which represent the amount of O₂ absorbed, are the means obtained from experiments replicated five or six times. The standard errors are given in brackets. The same intestinal preparation was used for a particular toxin, heated and unheated. Experimental details in the text.

Toxin	Succinate alone	Toxin added	Heated toxin added	Difference columns 3 and 4
<i>Cl. welchii</i> , type A	100 (3.39)	16 (2.68)	73.2 (3.06)	57.2
<i>Cl. welchii</i> , type C	100 (3.72)	34.6 (1.69)	66.6 (2.16)	32.0
<i>Cl. welchii</i> , type D	99.7 (0.87)	70.2 (2.2)	83.2 (3.01)	13.0
<i>Cl. oedematiens</i>	100.13 (4.85)	73.5 (4.87)	72.83 (4.73)	0.67
<i>Cl. septicque</i>	100.13 (2.34)	80.17 (4.44)	78.33 (3.11)	1.84
<i>Cl. tetani</i>	100 (1.35)	94.17 (3.67)	95.60 (2.78)	1.43
Broth ppt. control	99.9 (1.54)	86.44 (2.58)	88.94 (3.20)	2.50

toxins from *Cl. septicque*, *Cl. oedematiens* and *Cl. tetani* are unaffected by first heating the toxin but the inhibitions produced by the toxins from the three types of *Cl. welchii* are considerably lessened by pre-heating the toxin. This is further evidence suggesting that the inhibitory action is associated with the α toxin of *Cl. welchii*.

The effect of the addition of the toxin of Cl. welchii, type A, upon other oxidations by small intestine. The only substances which appear to be oxidized actively by aqueous suspensions of minced small intestine are succinate, *p*-phenylenediamine and dihydroxyphenylalanine (Table I). If the various toxins are added to mixtures of *p*-phenylenediamine and intestine in the micro-respirometer they all, including the broth precipitate control, inhibit the oxidation nearly 20 % but the toxins of *Cl. welchii*, type A, possesses a slightly more inhibitory action (*vide* Table VIII). The action of this toxin is significantly different from that of the broth precipitate control, but its magnitude is small in comparison with its inhibitory action on the succinate oxidation. Furthermore no inhibition significantly greater than that produced by the broth precipitate is obtained with the toxins of *Cl. welchii*, types C and D.

The addition of the toxin of *Cl. welchii*, type A, to dihydroxyphenylalanine (*M*/120) and minced small intestine diminishes the rate of oxidation to about 60 % of its normal value, an inhibition greater than that shown with *p*-phenylenediamine but only about half that registered with succinate (*cf.* Tables IX and II).

Table VIII. *Effects of bacterial toxins upon the aerobic oxidation of p-phenylenediamine in the presence of minced small intestine*

Each experiment was made in quadruplicate. Abbreviations are the same as for Table II, but pp.d. = *p*-phenylenediamine (*M*/54).

Toxin	pp.d.	pp.d. +BP	pp.d. + A	pp.d. + C	pp.d. + D	pp.d. +Oed.	pp.d. +Sept.	pp.d. +Tet.
No. of exps.	10	6	5	5	5	5	5	5
O ₂ uptake	100	82.78	72.58	86.37	81.3	79.23	82.15	82.66
s.e.	1.53	1.24	1.85	2.25	2.18	1.52	1.84	1.79
% inhibition	—	17.22	27.42	13.63	18.7	20.37	17.85	17.34
Values for <i>P</i> compared with:								
(a) Substrate control	—	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
(b) Broth ppt. control	—	—	<0.01	0.6-0.7	0.4-0.5	0.5-0.6	0.7-0.8	>0.90

Table IX. *The effect of the toxin of Cl. welchii, type A, upon the rate of oxidation of M/20 dihydroxyphenylalanine by minced small intestine*

The experimental detail is the same as that described for succinate except that, owing to the slower rate of oxidation, the figures given represent the O₂ absorbed over a period of 80 min. Control experiments with "broth precipitate" gave small inhibitions comparable with that reported for succinate. s.e. = standard error.

	O ₂ absorbed	s.e.	No. of exps.	No. of manometers
Substrate alone	100	2.12	5	16
Substrate + toxin	61.4	1.90	5	16

The effect of the toxin of Cl. welchii, type A, upon the aerobic oxidation of succinate by various tissues. Minced preparations of small intestine, skeletal muscle, heart muscle, liver, kidney and brain actively oxidize succinate in the Barcroft apparatus. The activity of heart muscle is very great, being 15 times greater than that of skeletal muscle. Despite their varying activities towards succinate the addition of the toxin of *Cl. welchii*, type A, to aqueous suspensions

Table X. *The effect of the addition of the toxin of Cl. welchii, type A, upon the aerobic oxidation of succinate by various tissues*

The results given are from single experiments replicated five times and are based on 40 min. readings, except for skeletal and heart muscle (20 min.). The experimental technique is described in the text. All inhibitions except that obtained in one of the brain experiments and that with brain and the broth precipitate control, are significant (i.e. *P* = <0.01). Three results are given with brain as they vary considerably from animal to animal. This variation does not occur with other tissues. The tissues were suspended in tap water. The standard errors are given in brackets.

Tissue	Dry wt. of tissue	Q _{O₂}	O ₂ uptake		% inhibition
			Succinate	Succinate + toxin or broth ppt.	
(a) With toxin:					
Intestine	13.83	11.76	100.11 (0.46)	15.79 (0.35)	84.32
Skeletal muscle	38.00	8.37	100.5 (4.74)	16.5 (2.75)	84
Heart muscle	2.40	126.25	100.25 (3.33)	29.2 (3.54)	71.05
Liver	16.50	11.64	100.33 (3.56)	38.25 (4.77)	61.08
Kidney	7.80	35.00	99.8 (2.85)	25.8 (2.06)	74.00
Brain	7.80	7.20	100.25 (5.12)	109.75 (2.56)	— 9.5
Brain	17.30	13.20	99.83 (3.71)	78.17 (5.41)	21.66
Brain	12.50	12.78	99.60 (2.18)	52.00 (4.04)	47.60
(b) With broth ppt.:					
Intestine	14.47	12.06	100 (1.16)	82.41 (1.04)	17.59
Brain	8.0	13.80	99.9 (1.28)	95.75 (2.54)	4.15

of these tissues greatly inhibits the rate of oxidation of succinate with each tissue except brain. The inhibition is generally between 60 and 85%, but with brain the results are indefinite although suggestive that some inhibition, less than that obtained with other tissues, occurs (Table X).

The effects of bacterial toxins upon a dried preparation of tissue enzymes. A dried preparation of "lactic dehydrogenase" was obtained from ox heart as described by Green & Brosteaux [1936]. This preparation was active to some extent with lactate and malate but it was much more active with succinate as its substrate. If the various bacterial toxins are added to mixtures of this enzyme preparation and succinate the rate of oxidation of the succinate is inhibited by the different toxins to an extent comparable with that shown with small intestine (cf. Tables II and XI). The toxins of *Cl. welchii* exert specific inhibitions greater than that

Table XI. *The effect of bacterial toxins upon a dried preparation of succinic dehydrogenase obtained from ox heart*

The abbreviations, experimental technique and method of presentation of results are similar to those described for Table II. A small amount of cytochrome *c*, prepared by the method of Keilin and Hartree [1937], was added to each cup. The number of experiments made was not large.

	S	S + BP	S + A	S + C	S + D	S + Oed.	S + Sept.	S + Tet.
No. of exps.	3	2	3	2	2	2	1	1
No. of manometers	10	6	10	6	6	6	3	3
O ₂ absorbed	100.22	82.00	19.64	36.16	57.50	83.00	87.33	75
s.e.	2.93	3.42	1.38	3.87	2.50	2.21	0.33	6.03
% inhibition	—	18.00	80.36	63.84	42.50	17.10	12.67	25.00
Values for <i>P</i> compared with:								
(a) Succinate control	—	<0.01	<0.01	<0.01	<0.01	<0.01	0.02–0.05	<0.01
(b) Broth ppt. control	—	—	<0.01	<0.01	<0.01	0.8–0.9	0.3–0.4	0.2–0.3

induced by the control broth precipitate. In one or two similar experiments carried out with this enzyme preparation but using lactate or malate as substrate no inhibition was produced by the presence of the toxin of *Cl. welchii*, type A. The rate of O₂ absorption with these substrates was only half that given with succinate and as the experiments have only been done twice their preliminary character must be emphasized.

The effect of the toxin of Cl. welchii, type A, upon succinic dehydrogenase. The experiments described in this paper suggest that the α toxin of *Cl. welchii* inhibits the oxidation of succinate by its dehydrogenase. If the activity of the

Table XII. *The effect of the toxin of Cl. welchii, type A, upon the anaerobic oxidation of succinate by its dehydrogenase*

The dehydrogenase preparations used were minced small intestine and a dried tissue product prepared from ox heart after Green & Brosteaux [1936]. The usual anaerobic methylene blue technique was followed except that Thunberg tubes with hollow stoppers were employed and the succinate and methylene blue were tipped into the enzyme and toxin mixture after 10 min. anaerobic incubation at 45°. Reduction times (RT) in min. and the relative velocities (V) obtained from the reciprocals of reduction times are shown.

Exp.	Small intestine		Dried preparation	
	RT	V	RT	V
(a) Enzyme alone	∞	0	∞	0
(b) Enzyme + toxin	36.4	27	∞	0
(c) Enzyme + succinate	14.5	69	19	106
(d) Enzyme + succinate + toxin	10.4	96	24	83
(b + c) – d	—	0	—	23
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enzyme in minced small intestine or in the dried tissue enzyme preparation be examined anaerobically in the presence of the toxin of *Cl. welchii*, type A, no marked inhibition is observed (Table XII). The addition of methylene blue, to act as an "oxygen carrier", to a mixture of small intestine, succinate and toxin in the Barcroft micro-respirometer should reduce the inhibition of the succinate oxidation by the toxin if the latter does not exert its effect upon the dehydrogenase. The results given in Table XIII show that in the presence of 1/3000 methylene blue the inhibition was reduced from 78 to 44 %.

Table XIII. *The inhibition of the aerobic oxidation of succinate by small intestine in the presence of the toxin of Cl. welchii, type A and methylene blue*

The O₂ absorbed in the presence of methylene blue (1/3000) was slightly more than without it, viz. 110 instead of 100. The experiment was made in quadruplicate. S=succinate, A=toxin of *Cl. welchii*, type A (0.4 mg.), MB=1/3000 methylene blue and S.E.=standard error.

	S	S + A	S + MB	S + A + MB
O ₂ absorbed	100	21.75	100	56
S.E.	1.58	0.48	1.58	2.97
% inhibition	—	78.25	—	44
% reduction of inhibition	—	—	—	43.8

The effect of the toxin of Cl. welchii, type A, upon the indophenol oxidase of small intestine. If the presence of indophenol oxidase in small intestine is tested for with Nadi reagent in the way described by Keilin [1929], a very strong positive reaction is obtained. This reaction does not develop in the presence of cyanide but it is practically unaffected by the addition of the toxin of *Cl. welchii*, type A. Similar results were obtained with yeast and with heart muscle suspensions.

DISCUSSION

The results reported in this paper show that those bacterial toxins which contain the α toxin of *Cl. welchii* exert a specific inhibitory action upon the aerobic oxidation of succinate by aqueous suspensions of various minced tissues, e.g. small intestine, skeletal muscle, heart muscle, liver and kidney. A similar effect is experienced with a dried enzyme preparation made from ox heart by the method of Green & Brosteaux [1936]. A few preliminary experiments suggest that the aerobic oxidation of *p*-phenylenediamine by small intestine or of lactate or malate by the dried enzymic product is not inhibited by this toxin. Some inhibition, although only half as much as that with succinate, of the aerobic oxidation of dihydroxyphenylalanine by small intestine is experienced in the presence of the toxin. A number of other toxins of the *Clostridium* group of bacteria do not show any inhibition greater than that induced by the addition of a similarly prepared precipitate from uninoculated broth. The inhibitory action of the toxins appears to be specific to the α toxin of *Cl. welchii* for no toxin without the α toxin produces effects greater than the broth precipitate control, the inhibitory action is destroyed by heating to 80° for 15 min. and it is largely neutralized by the addition of antisera containing antibodies for the α toxin.

In comparison with its toxicity to the live animal the action of the toxin upon this enzyme system of tissues is low, although this may be due in part to our experimental conditions. Working on a basis of the presence of 70 % water in a live guinea-pig, the presence of 0.01 mg. toxin of *Cl. welchii*, type A, per g. dry tissue is lethal, whereas for the production of an inhibition of 80 % of the activity of the succinate system 1 g. dry tissue (small intestine) requires the

presence of 29 mg. of the toxin. Nevertheless, as an inhibitor of enzymic activity the toxin is potent.

If the inhibitory action of the toxin is examined under anaerobic conditions it is found that no such marked inhibition of the activity of succinic dehydrogenase is depicted. Neither can any inhibitory action of the toxin towards the activity of the indophenol oxidase of small intestine be demonstrated. These results, together with the aerobic oxidation of succinate by small intestine in the presence of toxin and methylene blue suggest that the inhibitory action of the toxin is not exerted directly either upon the dehydrogenase or upon the oxidase but may be associated with an interference to some intermediate link, possibly with an inactivation of some "carrier catalyst".

It has been clearly demonstrated that the α toxin of *Cl. welchii* interferes with a respiratory mechanism associated with the aerobic oxidation of succinate present in various animal tissues. Whether the pathogenicity of *Cl. welchii* is associated in any way with this inhibitory action upon an important respiratory mechanism of tissues is not known, but it is clear from recent progress in our knowledge of the physiological action of certain vitamins, e.g. lactoflavin or ascorbic acid, that the absence of factors associated with respiratory mechanisms of cells can lead to grave disease. Interference with enzymic systems necessary to the supply of energy to tissues might readily lead to subnormality of function, which may open the way to the development of a more active pathological condition. In this way a particular bacterial toxin might facilitate the subsequent invasion of the body by the toxigenic organism itself or by some other organism present at the time.

SUMMARY

1. The toxins obtained from *Cl. welchii*, types A, C and D, inhibit the aerobic oxidation of succinate by aqueous suspensions of minced small intestine.
2. This inhibition is reduced to a considerable extent by the presence of antisera which neutralize the α toxin of *Cl. welchii*.
3. The inhibitory action of the toxins is greatly reduced by heating for 15 min. at 80°.
4. The inhibitory action appears to be due to the α toxin.
5. The toxins of *Cl. oedematiens*, *Cl. septicum* and *Cl. tetani* exert no action different from that induced by a precipitate prepared similarly from the broth medium.
6. This inhibitory action is not due to an effect upon either the succinic dehydrogenase itself or the indophenol oxidase, but may be due to an action upon some intermediate carrier catalyst at present unidentified.
7. It is suggested that the pathogenicity of *Cl. welchii* may be associated in part with this inhibitory action of its α toxin upon an important respiratory mechanism of tissues.
8. A brief discussion is given of the importance to invading bacteria of factors which will inhibit enzymic reactions of the host.

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