CCLXXIII. THE MECHANISM OF CATALASE INHIBITIONS.

BY HERMANN BLASCHKO.

*From the Physiological Laboratory, Cambridge.*¹

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THE experiments recorded in this paper represent an introduction to a study of the effect of catalase inhibition on tissue respiration. Before this problem could be approached, it was desirable to obtain information as to the mechanism of the inhibitory action of substances that are known to act more or less specifically on catalase. This paper deals mainly with the question as to whether the action of a given substance on the enzyme is reversible.

The two principal catalase inhibitors so far studied are cyanide and sulphide. These substances have not been included in the present work because (a) they exhibit such striking properties as inhibitors of cell respiration that they are not suitable for the final purpose of our investigation, and (b) it has already been proved that the action of both drugs is a reversible one. [Rona *et al.*, 1925. For full references see Euler, 1934.] The reversibility of these inhibitions can be demonstrated by bubbling air through solutions containing the poisoned enzyme. This method is obviously suitable only in the case of a volatile inhibitor. Another possible way of deciding whether an inhibition is reversible or not can only be used in heterogeneous systems: *e.g.* the reversibility of the cyanide inhibition of hydrogen peroxide decomposition by palladium black has been demonstrated by washing the catalyst and adding new substrate [Blaschko, 1926].

For the present work it was necessary to use a method that was applicable in the case of non-volatile substances acting in a homogeneous solution. The action of catalase was followed manometrically using the Warburg type of the simple Barcroft manometer. The reversibility of the inhibition was studied in specially made manometer flasks in which the concentration of the inhibitor could be decreased without any other variation which would affect the rate of the reaction.

Material and methods.

All experiments were carried out in a phosphate buffer solution of p_H 7.4. In a few preliminary experiments a crude extract of rat liver was used as catalase preparation, but most of the experiments were carried out with a purified preparation from horse liver for which the author is indebted to Prof. D. Keilin. Just before the experiment, 0.1 ml. of this preparation was diluted with 50 ml. 0.01 *M* phosphate buffer. The stock solution of hydrogen peroxide was Merck's perhydrol (about 9.5 *M*), of which 1.0 ml. was diluted with 11.5 ml. water. This dilute solution was also freshly prepared. For the measurement of enzyme

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activity, with and without inhibitor, the usual conical Warburg flasks with one side bulb were used. The flasks were filled as follows:

Main vessel	1.98 ml. 0.01 <i>M</i> phosphate buffer solution + 0.02 ml. catalase solution + 0.25 ml. water or inhibitor
Side bulb	0.25 ml. hydrogen peroxide solution.

The final concentrations were therefore:

Phosphate buffer	0.008 <i>M</i>
Hydrogen peroxide	0.076 <i>M</i>
Catalase	62500-fold dilution of the pure preparation.

The flasks were then attached to their manometers and placed in a bath kept at or slightly below room temperature (11–18°). The reaction was started by tipping the hydrogen peroxide into the main vessel from the side bulb. In a single experiment the reaction rates with different concentrations of a given inhibitor were measured and compared with the rate with the unpoisoned enzyme.

For the study of the reversibility of the inhibition, special flasks were made by Messrs W. Flaig and Sons, London. They are of conical shape and carry two side bulbs that branch off from a common stem (see Fig. 1). The contents of the two side bulbs can be mixed first and then later the mixture can be emptied into the main vessel. The experiments were carried out in the following way. Two flasks of the type described were prepared as follows:

Fig. 1.

Flask no. ...	1	2
Main vessel	1.80 ml. 0.01 <i>M</i> phosphate buffer + 0.225 ml. hydrogen peroxide solution + 0.225 ml. water	1.80 ml. 0.01 <i>M</i> phosphate buffer + 0.225 ml. hydrogen peroxide solution + 0.225 ml. inhibitor solution
Side bulb I	0.18 ml. 0.01 <i>M</i> phosphate buffer + 0.02 ml. catalase solution + 0.025 ml. inhibitor solution	— As in side bulb I of flask No. 1 —
Side bulb II	0.025 ml. hydrogen peroxide solution	As in side bulb II of flask No. 1

The reaction in each flask is started by mixing the solutions in the two side bulbs and after a suitable time, 5 to 10 min., the mixed content of the side bulbs is tipped into the main vessel. As can be seen from the data given above, the concentration of poison, equal for both flasks during the first reaction period, is maintained at the original level in flask No. 2, whereas in flask No. 1 a tenfold dilution of inhibitor takes place. In addition to this change in inhibitor concentration, an unavoidable dilution of the enzyme takes place, but under the conditions of the experiments this dilution has no influence on the reaction rate, a fact, for which flask No. 2 serves as a control. The data of the experiments given below show that the readings in flask No. 2 always give a smooth curve, without break at the point of dilution. As is well-known, the rate of the catalase reaction does not remain constant for long periods, and the experiments were therefore kept as short as possible.

Experiments.

The substances studied were in the first instance those catalase poisons that were likely to be useful as catalase inhibitors in tissues, but as the method described was found simple and gave definite results, it was applied to other catalase inhibitors the mechanism of which is still uncertain.

Hydroxylamine. The action of hydroxylamine on catalase was discovered by Jacobson [1892] and was later studied by Senter [1905]. More recently Yakushiji [1933] and Shibata and Yakushiji [1933] used hydroxylamine to prove their theory that the decomposition of hydrogen peroxide by catalase is part of the assimilation process in green plants, identical with the so-called Blackman reaction, which had already been compared with the action of catalase by Willstätter and Stoll [1918] and by Warburg and his collaborators [see Warburg and Uyesugui, 1924; Yabusoe, 1924; Warburg, 1925]. According to Shibata and Yakushiji, hydroxylamine inhibits photosynthesis in concentrations which have no effect on respiration.

Table I. *Inhibition of catalase by hydroxylamine hydrochloride.*

Temperature 16.2°.						
Flask no.	...	...	1	2	3	4
Hydroxylamine hydrochloride concentration			0	$10^{-6} M$	$10^{-5} M$	$10^{-4} M$
μ l. O ₂ liberated: 0–2.5 min.			141	51	9	1.5
2.5–5 „			62.5	40	10.5	1
5–7.5 „			44	29	9.5	2
7.5–10 „			—	25	9.5	2
Remaining activity as % of that in flask no. 1			First 2.5 min.	36	6.5	1
			First 5 „	45	9.5	1

Table II. *Reversal of hydroxylamine hydrochloride inhibition.*

Temperature 16.6°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 10 min.

Flask no.	...	...	1	2
Initial hydroxylamine concentration			$10^{-4} M$	$10^{-4} M$
Final hydroxylamine concentration			$10^{-5} M$	$10^{-4} M$
μ l. O ₂ liberated: 0–5 min.			0.75	1.5
5–10 „			0.75	2
10–15 „			16	1.5
15–20 „			16	3

Tables I and II give the results of my own experiments with hydroxylamine hydrochloride. Table I shows the influence of different concentrations of the drug on the rate of hydrogen peroxide decomposition and Table II demonstrates that the inhibition is a reversible one: after dilution the reaction rate is considerably greater than in the control in which the concentration of hydroxylamine has been maintained. The results are shown graphically in Figs. 2 and 3.

Hydrazine, phenylhydrazine. The inhibition of catalase by the latter substance has already been described [Loew, 1901; Yamasaki, 1921]. Tables III and IV show that both substances are much less powerful inhibitors than hydroxylamine. They both give reversible inhibitions, but as hydroxylamine seems to be the most suitable and the most active representative of this group of inhibitors, the experiments need not be reproduced *in extenso*.

Fig. 2.

Fig. 3.

Fig. 2. Hydroxylamine inhibition of catalase (see Table I).

Curve I. Without hydroxylamine hydrochloride.

Curve II. With $10^{-6} M$ hydroxylamine hydrochloride.Curve III. With $10^{-5} M$ hydroxylamine hydrochloride.Curve IV. With $10^{-4} M$ hydroxylamine hydrochloride.

Fig. 3. Reversibility of hydroxylamine inhibition of catalase (see Table II).

Curve I. Hydroxylamine concentration decreased from $10^{-4} M$ to $10^{-5} M$ at 10 min.Curve II. Hydroxylamine concentration maintained at $10^{-4} M$ .Table III. *Hydrazine sulphate inhibition of catalase.*

Temperature 11.2°.					
Flask no.	...	...	1	2	3
Hydrazine concentration			0	10 ⁻³ <i>M</i>	10 ⁻² <i>M</i>
μl. O ₂ liberated: 0 -2.5 min.			131.5	51.5	0.5
2.5-5 "			60.5	38	1.5
Remaining activity as % of			First 2.5 min.	39	0.5
that in flask no. 1			First 5 "	46.5	1

Table IV. *Phenylhydrazine hydrochloride inhibition of catalase.*

Temperature 16.8°.							
Flask no.	...	...	1	2	3	4	5
Phenylhydrazine concentration			0	10 ⁻⁵ <i>M</i>	10 ⁻⁴ <i>M</i>	10 ⁻³ <i>M</i>	10 ⁻² <i>M</i>
μl. O ₂ liberated 0 -2.5 min.			119	90	59.5	23	3.5
2.5-5 "			59.5	46.5	46	24	6
Remaining activity as % of			First 2.5 min.	75	50	19	3
that in flask no. 1			First 5 "	76	59	26	5

Sodium azide. The inhibition of catalase by azide has only very recently been discovered [Keilin and Hartree, 1934]. It is interesting to note that azide also inhibits slightly the catalytic decomposition of hydrogen peroxide by colloidal platinum [Oliveri-Mandalà, 1929; see also Audrieth, 1934]. The action of azide

is especially interesting in connection with the fact that azide also gives a definite methaemoglobin compound [Keilin, 1933].

Table V shows that sodium azide is a very powerful inhibitor of catalase. Under the conditions of our experiments only about one-fifth of the activity remains with a concentration of azide as low as $10^{-6} M$.

The action of sodium azide on catalase is completely reversible (see Table VI).

Table V. *Sodium azide inhibition of catalase.*

Temperature 12.2°.						
Flask no.	...	...	1	2	3	4
Azide concentration			0	$10^{-7} M$	$10^{-6} M$	$10^{-5} M$
$\mu l.$ O ₂ liberated: 0 - 2.5 min.			104.5	87.5	20.5	8.5
2.5- 5 "			57	54.5	16.5	6.5
5 - 7.5 "			43.5	36	13.5	5
7.5-10 "			33	31	11.5	6
Remaining activity as % of that			First 2.5 min.	84	19.5	8
in flask no. 1			First 5 "	88	23	11

Table VI. *Reversibility of sodium azide inhibition.*

Temperature 17.6°. Contents of side bulbs I and II mixed at 0 min.; contents of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	1	2
Initial azide concentration			$10^{-6} M$	$10^{-6} M$
Final azide concentration			$10^{-7} M$	$10^{-6} M$
$\mu l.$ O_2 liberated: 0 - 2.5 min.			35	35.5
2.5- 5 "			35	34
5 - 7.5 "			49.5	36.5
7.5-10 "			48	30

Monoethyl peroxide. That monoethyl peroxide is not a substrate for catalase action has been long known [Bach and Chodat, 1903]; that it is an inhibitor of catalase was shown by Stern [1932]. That monoethyl peroxide combines with methaemoglobin has been shown by Keilin and Hartree [1935], who studied the

Table VII. *Monoethyl peroxide inhibition of catalase.*

Temperature 14.7°.					
Flask no.	...	...	1	2	3
Ethyl peroxide concentration			0	$6 \times 10^{-4} M$	$6 \times 10^{-3} M$
$\mu l.$ O_2 liberated 0 - 2.5 min.			149	89.5	40.5
2.5- 5 "			93.5	61.5	44
5 - 7.5 "			64	47	42.5
7.5-10 "			—	40	36
Remaining activity as % of			First 2.5 min.	60	27
that in flask no. 1			First 5 "	62	35

properties of the compound. Table VII shows that monoethyl peroxide is not as active a catalase inhibitor as the substances previously studied. The inhibition is completely reversible (see Table VIII).

Table VIII. *Reversibility of monoethyl peroxide inhibition.*

Temperature 14.2°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	1	2
Initial ethyl peroxide concentration			$6 \times 10^{-3} M$	$6 \times 10^{-3} M$
Final ethyl peroxide concentration			$6 \times 10^{-4} M$	$6 \times 10^{-3} M$
$\mu\text{l. O}_2$ liberated:	0 - 5 min.		65.5	65
	5-10 "		84	62.5
	10-15 "		81	59.5
	15-20 "		71.5	43.5
	20-25 "		58.5	35.5
	25-30 "		48.5	30.5

Potassium chlorate, potassium perchlorate. The salt inhibition of catalase has often been studied [see Stern, 1932] and, since it did not appear likely that these inhibitions would prove useful for the purpose of our investigation on tissues, only a few substances were examined. Among these, chlorate and perchlorate have been found to be relatively powerful inhibitors [Senter, 1903; 1905]. The effects of different concentrations of these salts are given in Tables IX and X. The reversal experiment reveals an interesting difference in the mechanism of action of the two substances: the inhibition by chlorate is irreversible (Table XI), whilst that by perchlorate is reversible (Table XII).

Table IX. *Potassium chlorate inhibition of catalase.*

Temperature 15.1°.

Flask no.	...	...	1	2	3	4
Potassium chlorate concentration			0	$10^{-5} M$	$10^{-4} M$	$10^{-3} M$
$\mu\text{l. O}_2$ liberated:	0 - 2.5 min.		136	121	118	66
	2.5-5 "		77.5	64	58.5	23.5
	5-7.5 "		40.5	—	38	12.5
	7.5-10 "		—	—	33.5	11
Remaining activity as % of that in flask no. 1		First 2.5 min.	89	87	48.5	
		First 5 "	87	83	42	

Table X. *Potassium perchlorate inhibition of catalase.*

Temperature 14.9°.

Flask no.	...	...	1	2	3	4
Perchlorate concentration			0	$10^{-4} M$	$10^{-3} M$	$10^{-2} M$
$\mu\text{l. O}_2$ liberated:	0 - 2.5 min.		105	8	1	0
	2.5-5 "		145	14.5	2	0
	5-7.5 "		—	16	1.75	0
	7.5-10 "		114	17	1.75	0
Remaining activity as % of that in flask no. 1		First 2.5 min.	7.5	1	0	
		First 5 "	9	1	0	

Table XI. *Effect of dilution on the chlorate inhibition of catalase.*

Temperature 15.5°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	1	2
Initial chlorate concentration			$10^{-3} M$	$10^{-3} M$
Final chlorate concentration			$10^{-4} M$	$10^{-3} M$
$\mu\text{l. O}_2$ liberated:	0 - 2.5 min.		77	73
	2.5-5 "		27	29.5
	5-7.5 "		23.5	27.5
	7.5-10 "		17	16
	10-12.5 "		14	11.5
	12.5-15 "		12.5	9.5

Table XII. *Reversibility of perchlorate inhibition.*

Temperature 15.3°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 7.5 min.

Flask no.	...	...	1	2
Initial perchlorate concentration			$10^{-3} M$	$10^{-3} M$
Final perchlorate concentration			$10^{-4} M$	$10^{-3} M$
$\mu\text{l. O}_2$ liberated:	0 - 7.5 min.		4	7
	7.5-15 "		35	8.5
	15 -22.5 "		31	5

Mercuric chloride. Mercuric chloride inhibition of catalase is well known. It is only one of the many poisoning effects of this substance on enzymic reactions. Hata [1909] was the first to prove the reversible action of mercuric chloride on amylase and on proteolytic enzymes [see also Krebs, 1930; 1931]. The work of Hata makes it probable that the effect of mercuric chloride on catalase is reversible, but Stern assumes it to be irreversible. That the mercuric chloride inhibition is in fact reversible can easily be demonstrated [see Table XIV],

Table XIII. *Mercuric chloride inhibition of catalase.*

Temperature 16.2°.

Flask no.	...	...	1	2	3	4
Mercuric chloride concentration			0	$10^{-6} M$	$10^{-5} M$	$10^{-4} M$
$\mu\text{l. O}_2$ liberated	0 - 2.5 min.		130.5	99	64.5	2.5
	2.5- 5 "		67	40.5	35.5	2
	5 - 7.5 "		43	28.5	20	0.5
	7.5-10 "		37	23	23	2.5
	10 -12.5 "		29	18.5	17.5	2
Remaining activity as % of that in flask no. 1		First 2.5 min.	77	49	2	2
		First 5 "	71	50	2	2

Table XIV. *Reversibility of mercuric chloride inhibition.*

Temperature 16°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 10 min.

Flask no.	...	...	1	2
Initial mercuric chloride concentration			$10^{-4} M$	$10^{-4} M$
Final mercuric chloride concentration			$10^{-5} M$	$10^{-4} M$
$\mu\text{l. O}_2$ liberated:	0- 5 min.		1.5	2
	5-10 "		5	3
	10-15 "		12	7
	15-20 "		13	3
	20-25 "		17	5.5
	25-30 "		14.5	2
	30-35 "		12.5	3

but on comparing the data with those given in the preceding Table XIII, one must conclude that recovery is not complete, an irreversible effect is superimposed upon the reversible one, a mode of action comparable with the mechanism of the mercuric chloride inhibition of urease [Sumner and Myrbäck, 1930].

Resorcinol. For literature see Euler [1934]. The results of our experiments are given in Tables XV and XVI. Resorcinol inhibits slightly and its action is reversible.

Table XV. *Resorcinol inhibition of catalase.*

Temperature 11.7°.							
Flask no.	...	...	1	2	3	4	5
Resorcinol concentration			0	10 ⁻⁵ <i>M</i>	10 ⁻⁴ <i>M</i>	10 ⁻³ <i>M</i>	10 ⁻² <i>M</i>
μl. O ₂ liberated:	0 - 2.5 min.		142	91	36.5	5.6	-0.5
	2.5- 5 "		74.5	57	27	5.6	+0.5
	5 - 7.5 "		53.5	39	17.5	4.5	0
	7.5-10 "		47	33.5	14	4	-0.5
	10 -12.5 "		41	29.5	13	4.5	+1
Remaining activity as % of	First 2.5 min.		64	25.5	4	0	
that in flask no. 1	First 5 "		68	28.5	5	0	

Table XVI. *Reversibility of resorcinol inhibition.*

Temperature 12.2°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 10 min.

Flask no.	...	...	1	2
Initial resorcinol concentration			$10^{-2} M$	$10^{-2} M$
Final resorcinol concentration			$10^{-3} M$	$10^{-2} M$
μ l. O ₂ liberated:	0- 5 min.		8	7.5
	5-10 "		8.5	8
	10-15 "		29	8.5
	15-20 "		24.5	6.5
	20-25 "		18.5	5
	25-30 "		15.5	4.5

Benzidine, *p*-phenylenediamine, *m*-phenylenediamine. As the first two of these substances can act as substrates for peroxidase, it was interesting to see what influence they have on catalase. Tables XVII and XVIII show that they are both inhibitors of catalase, although not very powerful ones. Both inhibitions are reversible (see Tables XIX and XX). Since *m*-phenylenediamine, which does not act as a substrate for peroxidase, has an inhibitory action on catalase (Tables XXI and XXII), similar to that of *p*-phenylenediamine, it is unlikely that the inhibitory action of *p*-phenylenediamine depends on that part of the molecular structure which makes it a substrate for peroxidase.

Table XVII. *Benzidine inhibition of catalase.*

Temperature 14.0°.					
Flask no.	...	...	1	2	3
Benzidine concentration			0	$10^{-4} M$	$10^{-3} M$
μ l. O ₂ liberated:	0 - 2.5 min.		161.5	116	32.5
	2.5- 5 "		88	73	27.5
	5 - 7.5 "		—	—	16.5
	7.5-10 "		53.5	58.5	14
Remaining activity as % of that	First 2.5 min.		72	20	
in flask no. 1	First 5 "		76	22	

Table XVIII. *p*-Phenylenediamine inhibition of catalase.

Temperature 16.2°.					
Flask no.	...	...	1	2	3
<i>p</i> -Phenylenediamine concentration			0	10 ⁻³ <i>M</i>	10 ⁻² <i>M</i>
μl. O ₂ liberated:	0 - 2.5 min.		131.5	53.5	12.5
	2.5- 5 "		69	41.5	13.5
	5 - 7.5 "		38.5	34	12.5
	7.5-10 "		32.5	28	11
Remaining activity as % of that in		First 2.5 min.		41	9.5
flask no. 1		First 5 "		47	13

Table XIX. *Reversibility of benzidine inhibition.*

Temperature 15.0°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	...	1	2
Initial benzidine concentration				$10^{-3} M$	$10^{-3} M$
Final benzidine concentration				$10^{-4} M$	$10^{-3} M$
$\mu\text{l. O}_2$ liberated:	0	–	2.5 min.	23	23
	2.5	–	5 "	19	21
	5	–	7.5 "	40	24.5
	7.5	–	10 "	40.5	17.5
	10	–	12.5 "	37	18

Table XX. *Reversibility of p-phenylenediamine inhibition.*

Temp. 16.2°. Contents of side bulbs I and II mixed at 0 min.; content of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	...	1	2
Initial p-phenylenediamine concentration				$0.9 \times 10^{-2} M$	$0.9 \times 10^{-2} M$
Final p-phenylenediamine concentration				$0.9 \times 10^{-3} M$	$0.9 \times 10^{-2} M$
$\mu\text{l. O}_2$ liberated:	0	–	2.5 min.	12	8
	2.5	–	5 "	12.5	10
	5	–	7.5 "	24	11.5
	7.5	–	10 "	25	7
	10	–	12.5 "	17.5	8

Table XXI. *m-Phenylenediamine inhibition of catalase.*

Temperature 15.4°.

Flask no.	...	...	...	1	2	3
m-Phenylenediamine concentration				0	$10^{-3} M$	$10^{-2} M$
$\mu\text{l. O}_2$ liberated:	0	–	2.5 min.	144	107	5.2
	2.5	–	5 "	71.5	64	4
	5	–	7.5 "	47	47	5.8
	7.5	–	10 "	36.5	36	5.2
Remaining activity as % of that in flask no. 1			First 2.5 min.		67	4
			First 5 "		75	4.5

Table XXII. *Reversibility of m-phenylenediamine inhibition.*

Temperature 15.4°. Contents of the two side bulbs mixed at 0 min.; contents of side bulbs tipped into main vessel at 5 min.

Flask no.	...	...	...	1	2
Initial m-phenylenediamine concentration				$10^{-2} M$	$10^{-2} M$
Final m-phenylenediamine concentration				$10^{-3} M$	$10^{-2} M$
$\mu\text{l. O}_2$ liberated:	0	–	2.5 min.	3	5
	2.5	–	5 "	4.5	4.5
	5	–	7.5 "	70	3.5
	7.5	–	10 "	50.5	5
	10	–	12.5 "	32.5	3.5
	12.5	–	15 "	24.5	3

DISCUSSION.

The study of the mechanism of catalase inhibitors shows that all those substances that are known to give methaemoglobin compounds act as reversible inhibitors of catalase. To this group belong azide, monoethyl peroxide, sulphide and cyanide. That all these substances act as catalase inhibitors is certainly to be considered as a strong argument in favour of the conception of catalase as a haemin compound. Whether the action of hydroxylamine can be explained on similar lines still remains doubtful. One other substance which is known

to combine with methaemoglobin, *viz.* fluoride, was tested under the same conditions at the suggestion of Prof. Keilin, but was found to be ineffective as an inhibitor in concentrations as high as 10^{-3} *M*. It is interesting to note that Lipmann [1929] found a considerable inhibitory effect of fluoride on the hydrogen peroxide decomposition by inorganic iron. From the dissociation curve of fluoromethaemoglobin measured by Lipmann, the affinity of fluoride for methaemoglobin does not appear to be great and it is therefore possible that the conditions of our experiments in which the catalase is saturated with substrate are unfavourable for detecting slight inhibitory effects.

The reversible inhibition by mercuric chloride may serve to demonstrate that the reversibility, as such, is not a sufficient indication as to the point of attack of the inhibitor; as this effect of the poison is similar to its effect on other enzymes, it seems most likely that it acts on the non-haemin part of the enzyme molecule.

SUMMARY.

The action of catalase poisons is measured manometrically and a manometric method for the study of reversibility of catalase inhibitions is described.

The following substances are found to give reversible inhibitions: sodium azide, hydroxylamine, hydrazine, phenylhydrazine, monoethyl peroxide, potassium perchlorate, resorcinol, *p*-phenylenediamine and *m*-phenylenediamine. The mercuric chloride inhibition is partly reversible. Potassium chlorate gives an irreversible inhibition.

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