# CCL. THE DISTRIBUTION OF PEROXIDASE IN ANIMAL TISSUES.

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THE enzyme peroxidase has commonly been believed to be present in all animal tissues. It appears that no real evidence to this effect has yet been presented and the assumption seems to be based on early crude experiments and on the results of histological studies. The latter depend on the colours produced in fixed sections by strong hydrogen peroxide with benzidine, *p*-phenylenediamine and similar reagents. Such colours will, however, be given by any haematin compound, as for instance haemoglobin, in acid solution with strong $H_2O_2$. We would like to distinguish between the enzyme and other types of catalyst which promote oxidations by $H_2O_2$. The enzyme shows its full activity in neutral solution and at concentrations of $H_2O_2$ which are possible within the organism. Under these conditions the thermostable catalysts (see below) have less than one-ten-thousandth of the activity of a concentrated enzyme preparation.

The formation of $H_2O_2$ as a stage in the oxidation of organic substances is an integral part of Wieland's theory, and its formation has been demonstrated in various plant, bacterial and animal systems, as for instance in oxidations catalysed by the yellow ferment of Warburg. Though Elliott [1932, 1, 2] showed that peroxidase catalysed the direct oxidation of none of the common metabolites except tryptophan and phenolic compounds, he did not infer that the enzyme could have no general importance, rather suggesting that the metabolites may also need previous activation as in the cytochrome-oxidase system of Keilin.

We therefore felt it desirable to obtain an estimate of the peroxidase activity in the various tissues of normal animals and in tumours.

It seemed best to use for the estimation of peroxidase a method in which the conditions approached as closely as possible to those of Willstätter and Stoll [1918] in order that we could express results in terms of the unit of concentration "P.Z." defined by him and since used by various other workers.

In this method, as indeed in any method, it is necessary to have a definite concentration of hydrogen peroxide in the reaction mixture. Most tissues contain catalase which rapidly decomposes $H_2O_2$ and numerous methods were tried to remove catalase without damaging the peroxidase content. All such methods failed, and therefore a method was devised for compensating for the $H_2O_2$ decomposed.

Most of the experiments on the removal of catalase were tried on pig's spleen. Spleen was found to be an organ with comparatively high peroxidase content and though it contains a fair amount of catalase the amount was not so great as to vitiate entirely estimations of peroxidase activity; we were thus able to attempt to remove the catalase and test the effect of the treatment on the remaining peroxidase content.

We had hoped to be able to extract the peroxidase completely from the tissue, and to be able then to work with a clear centrifuged extract. However, though the tissue was minced to a fine paste in a Latapie mincer and then ground with sand and extracted with large volumes of water it was found that only about half the peroxidase activity went into solution, the remainder staying with the tissue debris. For instance, 9 g. of pig's spleen minced and ground with sand were shaken for an hour with 1800 ml. of distilled water. On taking 100 ml. of the suspension (500 mg. tissue) and making up to 500 ml. the usual estimation method gave 4·7 mg. purpurogallin. After centrifuging the suspension, 200 ml. of the centrifugate gave only 4·74 mg. purpurogallin. These amounts correspond respectively to P.Z. $9{\cdot}4 \times 10^{-3}$ and $4{\cdot}7 \times 10^{-3}$ on the wet weight of tissue. Neither alkaline phosphate solution nor 50 % glycerol removed the activity completely from the tissue. Extraction with glycerol, however, seems to bring out the peroxidase activity better than pure water, since the glycerol suspensions have higher activities than uncentrifuged aqueous suspensions. With a completely soluble milk peroxidase preparation the presence of glycerol was without effect, so the action of glycerol may depend on its viscosity maintaining a better dispersion of tissue particles.

This division of peroxidase into a dissolved and an undissolved fraction has previously been remarked by Willstätter and Pollinger [1928] in horseradish tissue, and with various other enzymes, and the terms desmo- and lyo-enzymes have been introduced to distinguish the two fractions.

Attempts were made to liberate the undissolved enzyme by drying the tissue and extracting the powdered residue. The minced tissue was dried with ether or acetone, or *in vacuo* over sulphuric acid. The treatment resulted always in the loss of about half the activity, and of the remainder only half could be got into clear solution. (No activity was found in the acetone or ether extracts.) Treatment of the tissue with alcohol always resulted in complete destruction of the peroxidase.

Undried tissue suspended in 50 % glycerol gave P.Z. $41{\cdot}0 \times 10^{-3}$.

The same after centrifuging gave P.Z. $29{\cdot}2 \times 10^{-3}$.

Acetone-dried tissue suspended in 50 % glycerol after centrifuging gave P.Z. $16{\cdot}9 \times 10^{-3}$.

In studying the possibility of removing catalase, clear centrifuged extracts were used. It is well known that alumina adsorbs catalase out of aqueous solution, while horseradish peroxidase is adsorbed on alumina from 50 % alcohol and eluted by pure water, and this difference seemed to offer a ready method for separating catalase. When, however, centrifuged aqueous extracts of spleen were treated with various amounts of Willstätter's alumina A or alumina C, the amount of adsorbent which would remove the catalase also removed the peroxidase practically completely.

Similarly calcium phosphate adsorbed a considerable amount of peroxidase as well as catalase. It seems possible that this adsorption of peroxidase on alumina, under conditions under which purified plant peroxidase is not adsorbed, may be due to the association of the enzyme with adsorbable proteins in the crude extract. Similarly the inactivation by alcohol may be due to a precipitation of the enzyme so occluded in denatured protein that it is unable to catalyse reaction in solution.

In the hope of liberating all the peroxidase in soluble form, attempts were made to digest away the proteins (by trypsin and papain) but these efforts failed owing to the rapid inactivation of peroxidase at temperatures of 25–35°.

Alcohol is without effect on the peroxidase of horseradish and is used for precipitating and drying preparations from this source. Alumina does not adsorb peroxidase from a crude aqueous extract of horse-radish; and drying such an extract *in vacuo*, and treating the dried solid with alcohol, do not affect its activity. It is clear that the proteins associated with the enzyme from different sources are very different.

*Method of estimation.*

The basis of the method was that of Willstätter and Stoll [1918]. This method consists simply in adding 10 ml. of 0·5 % $H_2O_2$ to two litres of water at 20° containing 5 g. of pure pyrogallol and a measured amount of the enzyme. The amount of enzyme is so chosen that 15–20 mg. of purpurogallin are formed in 5 minutes. At the end of 5 minutes the reaction is stopped with sulphuric acid, and the purpurogallin extracted with ether and estimated colorimetrically. Elliott and Keilin [1934] found the estimation satisfactory and more convenient using one fourth of the above quantities.

Since it seemed impossible to obtain the whole of the peroxidase in clear solution or free from catalase we used a fine suspension of the tissue in 50 % glycerol. During the whole five minutes of the reaction, under vigorous mechanical stirring, a solution of $H_2O_2$ of the strength calculated to maintain the initial $H_2O_2$ concentration was added.

The catalase activity of the suspension was first determined and the constant $k$ of the unimolecular decomposition of $H_2O_2$ obtained from the equation $k=\frac{2\cdot3}{t}\log_{10}\frac{a}{(a-x)}$ where $t$=time in minutes, $a$ and $(a-x)$ are the concentrations of $H_2O_2$ at the beginning and at time $t$ respectively. The velocity, $V$, of decomposition of $H_2O_2$ per minute by the catalase is then given by $V=kc$ where $c$ is the concentration of $H_2O_2$ (2·5 mg./100 ml.) required in the experiment.

The solution added per minute therefore should contain $V$ *plus* the required experimental concentration $c$, that is to say the concentration $C$ of $H_2O_2$ in the added solution is given by

$$C=c+V \text{ or } C=c\,(1+k).$$

In practice, owing to complex variations from a simple law with tissue suspensions, this calculation served only as a rough guide, and the actual amount of $H_2O_2$ necessary to add was usually somewhat greater.

It was necessary to avoid using too strong a solution of $H_2O_2$ lest local concentration of $H_2O_2$ might harm the peroxidase before it became well mixed; we therefore started the reaction in a volume of 250 ml. containing the proper concentrations of $H_2O_2$ and pyrogallol and during the five minutes 500 ml. of a solution containing 1·25 g. of pyrogallol and $H_2O_2$ of the necessary strength were added at the rate of 100 ml. per minute. Thus the mean volume of the reaction mixture throughout the experiment is 500 ml., the pyrogallol concentration remains the same and the $H_2O_2$ concentration is kept at the right level. To maintain a $p_H$ of 6 during the reaction, the mixture contained $M/200$ phosphate buffer.

The phosphate used had to be carefully freed from traces of copper and this was done by treating $KH_2PO_4$ solution with $H_2S$, filtering, boiling off excess of $H_2S$ and recrystallising twice from water. The buffer was then made up to $p_H$ 6 with pure NaOH solution. Untreated phosphate from Merck and Kahlbaum both form a yellow colour with pyrogallol and $H_2O_2$ quite rapidly. That this is due to traces of copper is suggested by the fact that HCN and $H_2S$ inhibit the action while pyrophosphate does not. A trace of copper in the phosphate could indeed be detected.

spectroscopically. Neither ferrous nor ferric iron in small amount would produce this reaction, while 0·01 mg. of copper sulphate in 500 ml. solution caused colour to be developed in a few minutes.

For the above reasons redistilled water was used throughout the work.

Since the experimental solutions contain a large amount of protein which causes obstinate emulsification in the ether extraction, it is necessary to stop the reaction with an acid protein precipitant, rather than $H_2SO_4$, and to filter before extraction.

Pyrogallol is easily oxidised to give a deep red colour, apparently by ether peroxides under the catalysis of iron or other complexes, and this is especially noticeable with tissue extracts and can seriously interfere with the colorimetric estimation of purpurogallin. To avoid this, ether of the best quality should be used; good salicylsulphonic acid dissolved in glass-distilled water is used as protein precipitant. For the filtration only an acid-washed paper such as Whatman No. 40 should be used.

Tissue peroxidase rapidly loses its activity in solution on standing, but it was found possible to keep organs moistened with Ringer solution or minced tissue in covered flasks for one or two days in the ice-box without appreciable loss.

The following is a detailed example of the procedure with a specimen of rat-liver.

The animal was killed by a blow on the head followed by bleeding from the neck. A weighed sample of the liver was dried in an oven at 100–110° to determine the dry weight/wet weight ratio. Then 4·4 g. of the liver were thoroughly ground with about 0·5 g. sand and shaken gently in a shaking machine for an hour with 80 times its weight of 50 % glycerol. To determine the catalase activity, 3 ml. of the suspension were mixed with 20 ml. of $M/20$ phosphate buffer $p_H$ 6·0 and 0·5 g. of pyrogallol[1] and made up to 200 ml. with redistilled water. To this 1·4 ml. (7·0 mg.) $H_2O_2$ solution were added and 10 ml. samples were withdrawn at the time intervals 0, 2, 4 and 6 minutes and run into 50 ml. flasks containing 5 ml. of 10 $N$ $H_2SO_4$. To each flask 4 ml. of 5 % KI were added, and after five minutes the iodine liberated was titrated with 0·005 $N$ thiosulphate. This gave the amount of $H_2O_2$ remaining after the various time intervals and from this the velocity constant of decomposition was calculated.

| Time | Titration (ml.) | $\frac{a}{a-x}$ | $k=\frac{2\cdot3}{t}\log_{10}\frac{a}{a-x}$ |
|---|---|---|---|
| 0 | 2·98 | — | — |
| 2 | 1·58 | 1·84 | 0·31 |
| 4 | 0·89 | 3·27 | 0·30 |
| 6 | 0·50 | 5·82 | 0·29 |

The constant for 3 ml. of suspension being 0·3, that for 200 ml. (the amount necessary for the peroxidase estimation) is 20. The amount of $H_2O_2$ necessary to add per minute to maintain a concentration of 2·5 mg./100 ml. is given by the expression $c(1+k)$ and is therefore $2\cdot5(1+20)=52\cdot5$ mg. or 263 mg. for 5 minutes.

Of the suspension 200 ml. were made up to 250 ml. with redistilled water containing 0·625 g. pyrogallol and 25 ml. of buffer. This mixture was put in a precipitating jar, adjusted to 20° and vigorously stirred with a mechanical stirrer throughout the experimental period. At zero time 1·25 ml. of 0·5 % $H_2O_2$ were

[1] The presence of pyrogallol depresses the activity of catalase considerably. It is therefore necessary to have its concentration in the catalase determination the same as in the peroxidase estimation.

added. At the same time 500 ml. of a solution containing 50 ml. buffer of $p_H$ 6, 1·25 g. pyrogallol and 320 mg. of $H_2O_2$, were run in steadily at the rate of 100 ml. per minute. (This amount of $H_2O_2$ is more than that theoretically required but was found actually to be necessary.) A constant rate of addition of this solution was obtained by letting the solution run in from a small funnel which was kept full, the stem of which had been adjusted to give the correct flow.

At the end of five minutes 35 ml. of 44 % sulphosalicylic acid were added to stop the reaction and precipitate the proteins. The fluid was filtered through Whatman No. 40 paper and extracted three times with a total of 200 ml. of good ether. The purpurogallin in the ether was estimated colorimetrically against a standard solution of purpurogallin in ether. In this case 2·52 mg. were formed,

$$\text{P.Z.} = \frac{\text{mg. purpurogallin}}{\text{mg. tissue}} = \frac{2{\cdot}52}{2500} = 1{\cdot}08 \times 10^{-3} \text{ on wet weight.}$$

The ratio wet weight/dry weight was found to be 3·08 whence

$$\text{P.Z.} = 3{\cdot}3 \times 10^{-3}$$

on the dry weight.

The $H_2O_2$ was estimated in a sample of the filtrate before extracting with ether and 2·1 mg./100 ml. were found present showing that the concentration of $H_2O_2$ has been successfully maintained near the initial 2·5 mg./100 ml.

As checks on the method the following experiments were tried.

First a preparation of milk-peroxidase [Elliott, 1932, 1] was mixed with a preparation of liver-catalase prepared according to Morgulis [1926].

The activity of the milk-peroxidase was determined in the ordinary way and found to be P.Z. 0·98.

Of the peroxidase 4·1 mg. were mixed with 8·0 mg. of the catalase preparation. The catalase activity of the mixture at 20° and in the presence of pyrogallol was found to be Kat. f. 160 on the dry weight of the preparation and on carrying out the peroxidase estimation on the mixture according to the method above described, the P.Z. on the dry peroxidase was found to be 0·96.

In a suspension of rat-liver tissue the catalase and peroxidase were estimated. Using 40 ml. of the suspension, 0·5 mg. of purpurogallin was formed in the estimation. The peroxidase estimation was then repeated, but to the suspension 4·0 mg. of milk-peroxidase of P.Z. 0·8 were added. Then 3·5 mg. of purpurogallin were formed, which means 3·0 mg. (3·5–0·5) of purpurogallin due to the added peroxidase. As 4·0 mg. of peroxidase give 3·2 mg. of purpurogallin, the result is in fair agreement with that expected.

Finally a suspension of liver tissue was allowed to stand for several days. The suspension then contained no peroxidase but contained catalase. On adding 3·3 mg. of milk-peroxidase of P.Z. 1·27 and carrying out an ordinary estimation, 4·3 mg. of purpurogallin were formed. This is again in good agreement with the 4·2 mg. of purpurogallin expected and shows that reducing substances and other materials in the suspension do not seriously interfere with the estimation.

## Results.

The distribution of catalase has been previously studied (see for instance Hawk [1911]) but we show here some of our own determinations of the catalase content of the various tissues so that the relative distributions of the two enzymes may be compared. These determinations are not directly comparable with catalase determinations made according to Euler and Jacobsen [1926] since we worked

at 20° instead of 0° and in presence of pyrogallol which has a strong inhibitory effect, nevertheless we have used their term for catalase activity, namely

$$\text{Kat. f.} = \frac{k\ (\text{constant of unimolecular reaction})}{\text{g. dry weight}}.$$

With all tissues tried it was found that after boiling the suspension the peroxidase activity was negligible.

Table I.

Figures in brackets are P.Z. $\times 10^3$ on wet weight.

| | | Peroxidase activity P.Z. $\times 10^3$ on dry weight | Catalase activity (in presence of pyrogallol see above) Kat. f. |
|---|---|---|---|
| Rabbit | spleen | 70 (17), 61, 113 | 4·6, 4·5 |
| | lung | 42 (8·4) | Trace |
| | kidney cortex | 4·6 (1·1), 3·7 | 23·0 |
| | liver | 7·3, 2·4, 8·7 (2·5) | 3·6, 7·2 |
| | brain | Trace | None |
| | adrenal | None | 3·5 |
| | muscle | Trace | None |
| | skin | None | None |
| | red bone marrow | 7·5 (5·7) | 2·0 |
| | serum | None | — |
| Rat | spleen | 105 (24), 70, 100*, 221* | 3·4, 5·5, 2·9*, 9·0* |
| | lung | 83 (17), 31 | 2·2, 1·9 |
| | whole kidney | 2, 9·5 (1·9), 4·0*, 3·5* | 8·8, 16·7, 5·6*, 10·3* |
| | liver | 3·3, 4·1, 4·6* (1·4), 2·8* | 26·5, 42·1, 17·0*, 17·3* |
| | mammary gland | None | None |
| | sarcoma (Philadelphia No. 1) | 1·8 (0·3), 1·3 | Trace |
| | carcinoma (Walker No. 256) | Trace | Trace |
| Chick embryo (8 day) | | Trace | Trace |
| Horse melanotic sarcoma | | 7·0 | 2·0 |
| Cow's milk | | (22) | — |
| Cow's colostrum | | 9·0 (3·2) | — |

* These estimations were made on healthy organs of tumour-bearing animals.

Remembering that Willstätter and Pollinger [1923] have obtained preparations with activity up to P.Z. 4700, it is seen from Table I that in no tissue tried is there any remarkably high peroxidase activity. Though the activity in any organ of different individuals varies considerably, the spleen appears to have the highest peroxidase content with a P.Z. of about 0·1, and lung the next highest. Kidney and liver follow but are much less active, while brain and muscle are practically inactive. In two types of malignant tumour activity seems absent while in a melanotic sarcoma there was some peroxidase.

The following consideration is of some interest. The activity of rabbit spleen is about P.Z. $70 \times 10^{-3}$, *i.e.* 1 mg. dry weight of tissue produces about 0·07 mg. purpurogallin in 5 minutes. From the formula, $C_{11}H_5O_8$, of purpurogallin it is calculated that $5{\cdot}4 \times 10^{-3}$ mg. of $H_2O_2$ must be activated to oxidise pyrogallol to this amount of purpurogallin. If tissue oxidations follow the Wieland formula completely ($AH_2 + O_2 = A + H_2O_2$) then for 22400 ml. of $O_2$ uptake 34 mg. $H_2O_2$ should be formed. The oxygen uptake rate of rabbit spleen is roughly 10 ml. per mg. dry weight per hour. If all this oxygen formed $H_2O_2$ there would be produced 34/22400 mg. $H_2O_2$ per hour, or in 5 minutes, $1{\cdot}25 \times 10^{-3}$ mg. Hence it appears that there is more than enough peroxidase activity in spleen to use all $H_2O_2$ formed supposing all oxygen uptake followed the Wieland mechanism. In liver

and kidney there would seem to be rather less than enough activity (one-third of that required) to account for all $O_2$ uptake proceeding through $H_2O_2$. However, it must be remembered that there is no reason to suppose that substances in the cell, possibly previously activated by other enzymes, would be oxidised by $H_2O_2$ and peroxidase at the same rate as is pyrogallol in our experimental conditions. The above consideration merely shows that in spite of low activity, such a mechanism is not disproved in the case of some organs. Nevertheless it seems to us most unlikely that peroxidase plays any large part in animal tissue oxidations, especially in view of the perfusion experiments in the next section.

*"True" peroxidase and haemoglobin derivatives.*

Willstätter and Pollinger [1928] found that oxyhaemoglobin from various animals had a peroxidase activity of P.Z. 0·06 to 0·13 under the normal estimation conditions. This value is considerably higher than that found in many organs, so it seemed possible that the activity found by us was due to haemoglobin in the tissues. We have attempted to check this possibility by perfusing organs to render them as free as possible from blood. Rabbits were anaesthetised with amytal, and warmed Ringer-Locke solution containing heparin was run in through the jugular vein while the animal was bled from the carotid artery, and the heart was massaged when it ceased beating. Finally the organs were separately perfused by syringing Ringer solution into main vessels. After this procedure the lung became almost pure white, the liver and kidney very much paler, but the spleen could not be very successfully perfused. Stained sections of the organs were prepared and showed that the tissues had been washed free of red blood corpuscles, except in the spleen where some corpuscles still remained. The experiment was tried on three animals and the following results obtained (Table II).

Table II. *Peroxidase activity of perfused rabbit organs.*

| | P.Z. $\times 10^3$ | | |
|---|---|---|---|
| Spleen | — | 29 | 88 |
| Lung | 50 | 52 | — |
| Kidney | — | Trace | Trace |
| Liver | — | Trace | Trace |

While the activity in kidney and liver appears to be almost completely removed, the activity of lung seems unaffected even though perfusion must have removed practically all haemoglobin. All the activity of spleen could certainly not be accounted for by haemoglobin since the activity of this tissue is of the same order as that of the pure pigment.

Since other haematin derivatives are known to be present in the cell a survey of the activity of various haematin compounds was made. After the experimental 5 minutes the protein was precipitated with salicylsulphonic acid, as in other experiments. The following summarises our findings.

*Alkaline haematin.* Haemin crystals were dissolved in a minimum amount of dilute alkali and the activity of the solution estimated in the usual way at $p_H$ 6; found P.Z. $125 \times 10^{-3}$ on the haematin, or P.Z. 1·57 on the haematin Fe.

*Pyridine haemochromogen.* Some of the above solution was reduced with a trace of sodium hydrosulphite[1] and pyridine added; found P.Z. $393 \times 10^{-3}$ on the haemochromogen, or P.Z. 4·5 on the haematin Fe.

*Nicotine haemochromogen.* Nicotine was added instead of pyridine; found P.Z. $232 \times 10^{-3}$ on haemochromogen, or P.Z. 4·2 on the haematin Fe.

[1] The hydrosulphite did not affect the $H_2O_2$ concentration appreciably.

*Oxyhaemoglobin.* Freshly prepared crystalline oxyhaemoglobin was kindly given us by Dr W. C. Stadie, found P.Z. $53 \times 10^{-3}$ on the haemoglobin, or P.Z. 16 on the haemoglobin Fe.

*CO-haemoglobin.* Carbon monoxide was prepared from formic acid and $H_2SO_4$, washed through NaOH solution and water and bubbled through the haemoglobin solution before and during the experiment; found P.Z. $66 \times 10^{-3}$. There is thus no inhibition by CO of the catalytic effect of haemoglobin. Elliott and Sutter [1932] found that CO does not affect other peroxidase preparations.

*Methaemoglobin.* Haemoglobin solution was treated with potassium ferricyanide and the excess salt dialysed away in the ice-box; found P.Z. $95 \times 10^{-3}$.

*Kathaemoglobin.* Methaemoglobin solution was shaken with a little chloroform; found P.Z. $118 \times 10^{-3}$.

*Haemochromogen.* Haemoglobin solution was treated with alkali and warmed, then sodium hydrosulphite was added; found P.Z. $57 \times 10^{-3}$ on the haemoglobin.

*Acid haematin.* Haemoglobin solution was treated with acetic acid and boiled, cooled and neutralised with NaOH. Though the pigment remained in fine suspension all activity was destroyed.

The activity of oxyhaemoglobin on standing in the ice-box for a week or more increases somewhat, probably owing to slow denaturation with the formation of kathaemoglobin. The following results were obtained with preparations from older haemoglobin; oxyhaemoglobin P.Z. $67 \times 10^{-3}$, methaemoglobin P.Z. $90 \times 10^{-3}$, kathaemoglobin $104 \times 10^{-3}$.

A small amount of concentrated cytochrome C solution kindly given to one of us by Prof. D. Keilin was found to have negligible peroxidase activity ($<$ P.Z. $20 \times 10^{-3}$) according to our estimation method.

## Discussion.

There is in horseradish and other plants a peroxidase enzyme which it is possible to concentrate in very active form. A similar enzyme appears to be present in milk, since Elliott [1932, 1] was able to obtain from milk a product with P.Z. as high as 4·7 although the preparation must have consisted largely of inactive lactalbumin. To what extent such an enzyme accounts for the small activity of animal tissues is difficult to decide. The criterion of thermolability hardly applies, since though activity in tissues is destroyed by boiling, a haemoglobin solution on boiling will coagulate and lose its activity. Even without coagulation, heat can cause inactivation as in the case of the acid haematin solution mentioned above. Of all the haematin compounds tested, only the pyridine and nicotine haemochromogens have activities higher than the haemoglobin derivatives (though the activity per unit of iron is actually lower), and these are not probable cell constituents. Lung and spleen do not consist almost entirely of haemoglobin substances, so it seems likely that there is in these organs some "enzyme" peroxidase. The activity of kidney and liver is lost on perfusion and so may be all due to haemoglobin. The "enzyme" peroxidase may itself be a special haematin compound though the work of Elliott and Keilin [1934] makes this doubtful.

## Summary.

1. Tissue peroxidase occurs in two fractions, one soluble in water and another associated with insoluble material.

2. Various attempts to remove catalase without affecting the peroxidase in solutions and suspensions failed.

3. A method for estimating peroxidase in the presence of catalase is described.

4. The peroxidase activity of various tissues has been determined. It is highest in the spleen and lung; liver and kidney contain a little. In most other tissues it is negligible.

5. The peroxidase activity of various haematin and haemoglobin derivatives has been estimated.

6. Perfusion removes the activity from liver and kidney; the activity therefore appears to be due to haemoglobin in blood corpuscles. In spleen and lung it is likely that there is some true peroxidase besides the activity due to haemoglobin and derivatives. It does not seem probable that peroxidase plays an important part in oxidations in any organ except possibly in lung and spleen.

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