This inhibitory compound could well be a new tricarboxylic acid containing six carbon atoms, such as oxalomalic acid. It seems very likely that such a compound, even at very low concentrations, could compete with the enzymic systems related to citrate oxidation.

The existence of the inhibitory effect of glyoxylate on the tricarboxylic acid cycle appears to be of great interest if we consider that glyoxylate has recently been shown to take part in many metabolic transformations involving carbohydrates, fats and protein derivatives. The fact that small amounts of glyoxylate in the presence of oxaloacetate will inhibit citrate oxidation strongly indicates that in the cells under physiological conditions, glyoxylate, however formed, must be promptly removed to avoid the inhibitory effects described above. On the other hand, glyoxylate could inhibit the normal rate of oxidations and might be connected with the widely observed oxidative impairment of cancer tissues (see Warburg, 1956; Weinhouse, Warburg, Burk & Schade, 1956; Ruffo, Capobianco & Adinolfi, 1956*a, b*), which has not yet been adequately explained.

SUMMARY

1. The effect of low concentrations (0.001M) of glyoxylate on the oxidative rate of the main intermediates of the tricarboxylic acid cycle was investigated in respiring rat-liver homogenates.

2. The addition of glyoxylate had little effect on the endogenous oxygen uptake of the homogenate, but produced a small inhibition of the oxidative rate with citrate, D-isocitrate, cis-aconitate, α -oxoglutarate, succinate, fumarate, malate and pyruvate, and a small increase in the accumulation of citrate. A very marked inhibition of the uptake of oxygen, and a large accumulation of citrate, occurred when oxaloacetate was the substrate.

3. The oxidation of citrate was inhibited either by glyoxylate alone, after incubation for 20 min.,

or by glyoxylate plus oxaloacetate, after incubation for 5 min. Both these results indicate that oxaloacetate was necessary for obtaining maximal rates on inhibition.

4. The dependence of citrate accumulation on oxaloacetate and glyoxylate suggests that the two substances react together to form an inhibitor of citrate metabolism. This would account for both citrate accumulation and the depressed oxygen uptake.

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The Relationship between Catalase and Haemoglobin in Human Blood

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There is insufficient evidence so far to indicate the biological function of catalase, but there is indication that its presence in the living cell is protective against the toxic effects of hydrogen peroxide which may be produced as a by-product during the various oxidative enzymic reactions that occur in living organisms. (Keilin & Hartree, 1945).

Catalase, which like haemoglobin is a haematin-

protein complex, is found principally in the red-blood cells and liver cells and is produced in the marrow and liver cells (Theorell, 1951). Its presence in plasma is probably due to haemolysis (Perlmann & Lipmann, 1947).

Interest in a possible role for hydrogen peroxide in the catabolism of haemoglobin was initiated by Bingold (1935), who found that haemoglobin could

be oxidized by hydrogen peroxide with resulting splitting of the tetrapyrrolic haematin ring into two dipyrrolic compounds, which then could be reduced to pink compounds—the pentdyopent reaction. It was later found, however, that this reaction could be obtained with other tetrapyrroles such as bile pigments.

Lemberg & Legge (1949) found that, under conditions approximating to those found in the circulating red cell, haemoglobin is converted into choleglobin (an iron-containing tetrapyrrole-globin complex with the tetrapyrrole ring modified) by coupled oxidation with ascorbic acid in the presence of oxygen. The ascorbic acid acts as a hydrogen donor, and an intermediary haemoglobin-hydrogen peroxide complex is formed. They also found that choleglobin formation in mammalian red cells is increased when the cells are haemolysed, and correlated this increase with a fall in catalase concentration that occurs with loss of stroma of the red cells.

In a review of some haemolytic mechanisms, Ponder (1951) has suggested that the presence of catalase in the red cell may be important in relation to red-cell ageing, since choleglobin-containing red cells are abnormally fragile, osmotically and mechanically. Like Lemberg & Legge, he considers it possible that catalase helps to preserve the red cell by reversing the change of haemoglobin to choleglobin at the intermediate haemoglobin-hydrogen peroxide stage, and quotes Foulkes & Lemberg (1949), who state that mammalian red cells rich in catalase have a longer life span than avian red cells, which have a low concentration of catalase. Keilin & Hartree (1945), however, have found that hydrogen peroxide which was being continuously generated in a system containing glucose oxidase produced small amounts of choleglobin and methaemoglobin from oxyhaemoglobin despite the presence of catalase.

Because of the common haematin prosthetic group of haemoglobin and blood catalase, their common distribution and common site of manufacture, and because of this possible protective effect of catalase for haemoglobin, it was decided to investigate the quantitative relationships between them in health and disease, especially anaemia.

Catalase acts by accelerating the decomposition of hydrogen peroxide into water and oxygen and its activity can therefore be estimated manometrically (e.g. Perlmann & Lipmann, 1947), or titrimetrically (e.g. Euler & Josephson, 1927; Bonnichsen, Chance & Theorell, 1947). In these methods, the velocity constants for the reactions were determined and used to estimate the activity of the catalase preparations by substitution in the formula $\text{Kat.f.} = k/(\text{g. of catalase protein in 50 ml. reaction mixture})$, where k is the reaction-velocity

constant and Kat.f. (Euler & Josephson, 1927) is the measure of purity of the preparation. Herbert & Pinsent (1948) found that crystalline human-erythrocyte catalase had a Kat.f. value of 63 000.

Euler & Josephson used dilute solutions of catalase and hydrogen peroxide and took readings of the resulting reactions at 3 min. intervals for 12 min. The reaction-velocity constants of each reading were calculated. These decreased in a linear fashion with time, since catalase is progressively destroyed during the reaction, so that the results had to be extrapolated to zero time, when no catalase had yet been destroyed. Bonnichsen *et al.* modified this method, by increasing the speed of the reaction with much larger concentrations of the enzyme, and took several readings during a period of 1 min.; the reaction-velocity constant then did not vary with time.

Using a manometric method, Perlmann & Lipmann (1947) also determined the Kat.f. value for their enzyme preparation obtained from blood, and expressed their results as a percentage of a 'pure' preparation of erythrocyte catalase of Kat.f. value 43 000. Theorell (1951) and Beers & Sizer (1953) have criticized the manometric procedure as not suitable for kinetic studies of catalase.

In the above methods the authors had attempted to obviate the destructive effect of the substrate upon the enzyme. They used an excess of enzyme so that all the substrate would have been destroyed had the reactions gone to completion, and assumed these to be of the first order. The timing of the readings had to be accurate, so that the necessary manipulations became difficult if many samples were to be examined.

The method used in this study employs a different principle. The destructive effect of the substrate is utilized so that all the enzyme is destroyed before complete decomposition of the hydrogen peroxide is obtained. The reaction, which under these conditions is not of the first order, is allowed to proceed to completion so that time is no longer a critical factor and the residual hydrogen peroxide is estimated titrimetrically. It is shown that the amount of the substrate utilized is directly proportional to the initial enzyme concentration, and it is easy in this way to compare the activities of different samples. To satisfy these conditions, the concentration of the enzyme must be considerably less than hitherto used.

It was found that haemoglobin and catalase are present in the red cell in a fairly constant proportion, irrespective of the severity of anaemia.

MATERIALS

Apparatus. A water bath at 37° and a rack for tubes, flat-bottomed glass tubes (5.5 cm. × 1.5 cm.) and a Conway microburette fitted with a mixing air bubbler (i.e. a small

pump passing a stream of air bubbles through the sample being titrated to ensure rapid mixing of the samples and the titrating fluid) were used.

Reagents. Potassium permanganate soln. (0.1 N); 0.02 M-phosphate buffer, pH 6.8; 2N- H_2SO_4 . AnalaR hydrogen peroxide solution ('90-100 volumes') was used for preparation of the substrate solutions by dilution with the phosphate buffer.

All the glass-ware was washed in hot soapy water and rinsed in several changes of water before drying in a hot-air cupboard. The use of acid-dichromate cleansing solution was found to be unsatisfactory owing to the difficulty of removing all traces of this solution, which then reacted with the hydrogen peroxide and so gave false measurements.

The blood under investigation was collected by one of two methods: either by skin prick from the finger tip or lobe of the ear for immediate assay, or by venepuncture if there was likely to be any delay before it could be examined. In the latter case the blood was kept fluid with Wintrobe's oxalate mixture (Whitby & Britton, 1950) and showed no appreciable loss of activity even if stored up to 3 weeks at 4°.

Once the blood had been diluted to the desired concentration, it was necessary to determine its activity as quickly as possible because, after dilution, the catalase activity decreased at a rate of about 5%/hr

METHODS

Haemoglobin estimation. The oxyhaemoglobin method described by Whitby & Britton (1950) was used except that 50 μl . of blood was diluted in 7 ml. of 0.04% (v/v) aq. NH_3 soln.

Whole-blood protein estimation. This was done with the micro-Kjeldahl-nesslerization method as described by Varley (1954).

Estimation of catalase activity. Whole blood (50 μl .) was laked in 7 ml. of 0.04% (v/v) aq. NH_3 soln. and the haemoglobin content estimated. The white cells, which contain peroxidase, were then removed by centrifuging. A solution containing 5 μg . of haemoglobin/ml. was then prepared by diluting with the buffer solution. Portions (2 ml.) were then placed in two tubes, which were transferred to a water bath at 37° for 5 min. to attain water-bath temperature. A volume (1 ml.) of 0.03 M-hydrogen peroxide soln., previously warmed to 37° in the water bath, was then added to each tube and also to two 'blank tubes' containing 2 ml. of phosphate buffer only. These 'blank tubes' were used to determine the initial amount of hydrogen peroxide added. The tubes were allowed to remain in the water bath for 75 min., when the reactions were complete. Sulphuric acid (2 ml. of 2 N soln.) was added to each tube and the residual hydrogen peroxide titrated with 0.1 N- KMnO_4 with a Conway microburette (1 ml. of 0.1 N- $\text{KMnO}_4 \equiv 560 \mu\text{l}$. of O_2 at s.t.p. derived from the H_2O_2 soln.). Because of the low concentrations of blood used, the amount of KMnO_4 reacting with the protein, or with the oxalate anti-coagulant, could be neglected.

The results were expressed as μl . of oxygen at s.t.p. evolved from the hydrogen peroxide during the reaction. This figure was obtained by converting the difference of the 'blank' and final titrations into volumes of oxygen, and was referred to as the number of units of catalase activity/10 μg . of haemoglobin.

To ensure that the loss of hydrogen peroxide that occurred was due to its breakdown into water and oxygen, and not to some other possible peroxidative or additive reaction, duplicate measurements were carried out on some of the blood samples with a manometric technique. The same quantities and dilutions of reagents were used in a Warburg manometric apparatus, and the total quantity of gas evolved, after the reaction had ceased, was estimated. Provided that the flasks were not shaken, the oxygen evolved was equivalent to the amount of hydrogen peroxide destroyed in the titrimetric method, and this was further confirmed by estimating the residual hydrogen peroxide in the manometric flasks.

During this experiment it was found that if the manometric flasks were shaken in the usual way, there was a considerable reduction in the amount of oxygen evolved and an equivalent reduction in the quantity of hydrogen peroxide destroyed. The effect of shaking on the reaction mixture is being further investigated, as it seems likely that this is the reason for the discrepancies observed in the manometric procedures by Theorell (1951) and Beers & Sizer (1953).

RESULTS

Fig. 1 shows that the reaction was completed in 60 min., although unchanged hydrogen peroxide was still present at this time; no further decomposition occurred even after 3 hr.

Fig. 2 shows that so long as there is incomplete breakdown of substrate at the end of the reaction, there is a linear relationship between blood dilutions, i.e. the catalase content, and the amount of hydrogen peroxide finally destroyed. Furthermore, there is a maximum quantity of substrate that can be decomposed by a given amount of catalase under these conditions.

From these results, it was found convenient to use 0.03 M-hydrogen peroxide solution, giving a final substrate concentration of 0.01 M, a final blood dilution of 10 μg . of haemoglobin/3 ml., and to allow at least 75 min. to elapse before estimating the residual hydrogen peroxide.

Repeated tests on one sample over several days showed an experimental error of just under 6%. Tests were also carried out at irregular intervals on one of the normal subjects, and the results always fell within a range of 10%, indicating a fairly constant figure for each subject.

It was desirable to calculate from the results obtained by this method the activity in terms of Kat.f., but since these were measured and calculated under different conditions from those of the present method, an analysis of the kinetics of the reactions taking place is necessary.

Curve A of Fig. 3 shows the logarithm of the concentration of undecomposed substrate plotted against time, from the results presented in Fig. 1. Curve A is not linear so that the overall reaction is not of the first order.

Fig. 1

Fig. 2

Fig. 1. Reaction of blood catalase with excess of hydrogen peroxide. Results of a timed experiment with a series of identical blood and substrate samples. The specimen of blood was diluted to contain $5 \mu\text{g.}$ of haemoglobin/ml. and the substrate concentration added was 0.02 M (1 ml. of 0.02 M -hydrogen peroxide solution = $224 \mu\text{l.}$ of available oxygen at s.t.p.). The horizontal broken line represents the available oxygen added to the enzyme.

Fig. 2. This composite curve represents the results of three experiments, allowed to proceed to completion, with three concentrations of added substrate [0.02 , 0.04 and 0.06 M -hydrogen peroxide solutions (i.e. 224 , 448 and $672 \mu\text{l.}$ of oxygen at s.t.p. respectively)] and three concentrations of the same blood sample (5 , 10 and $15 \mu\text{g.}$ of haemoglobin/ml.). The horizontal broken lines show the total available added oxygen for each experiment.

Fig. 3

Fig. 4

Fig. 3. The logarithm of substrate concentration left at different times is plotted against time, and is derived from the results shown in Fig. 1. In curve *A*, the zero-time concentration of substrate is taken as the concentration of the added hydrogen peroxide. Curve *B* is constructed on the assumption that the zero-time concentration was the amount of substrate finally destroyed.

Fig. 4. Reaction velocities obtained from a series of experiments allowed to proceed for 3 min. before being stopped with 2 ml. of 2 N -sulphuric acid. The blood sample used for Figs. 1 and 3 was used and diluted to $5 \mu\text{g.}$ of haemoglobin/ml. The left-hand figure is a detail from the first part of the right-hand figure. With this particular blood sample the maximum added substrate concentration that could be fully decomposed was 0.014 M (i.e. an activity of 160 units).

It is clear that the overall reactions between catalase and hydrogen peroxide under the conditions of these experiments involves not only the decomposition of the substrate but also the destruction of the enzyme itself.

Suppose the enzyme is destroyed in a first-order reaction, i.e. $-\frac{dE}{dt} = k_4 E$, where E is enzyme concentration at time t . Therefore

$$E = b e^{-k_4 t}, \quad (1)$$

where b is initial enzyme concentration. Now suppose that the enzyme reaction obeys the Michaelis & Menten (1913) law, i.e.

$$\frac{dx}{dt} = \frac{k_3 E(a-x)}{K_m + (a-x)}, \quad (2)$$

where a is initial substrate concentration, x is substrate decomposed at time t , K_m is the Michaelis constant.

$$\text{Therefore } \frac{dx}{dt} = \frac{k_3 b e^{-k_4 t}(a-x)}{K_m + (a-x)}$$

by substituting (1) in (2). Therefore

$$\int \frac{K_m + (a-x)}{(a-x)} dx = k_3 b \int e^{-k_4 t} dt,$$

$$\text{i.e. } x - K_m \ln(a-x) = \frac{-k_3}{k_4} b e^{-k_4 t} + C.$$

Now, $x=0$ when $t=0$, so that C can be determined. Therefore

$$x + K_m \ln \frac{a}{a-x} = \frac{k_3}{k_4} b (1 - e^{-k_4 t}), \quad (3)$$

$$\text{therefore } b = \frac{x + K_m \ln \frac{a}{a-x}}{\frac{k_3}{k_4} (1 - e^{-k_4 t})}.$$

If t is large, this becomes

$$b = \frac{k_4}{k_3} \left[x + K_m \ln \left(\frac{a}{a-x} \right) \right].$$

Now, if x is small compared with a ,

$$b = \frac{k_4}{k_3} x,$$

$$\text{i.e. } b = Kx \text{ (where } K = k_4/k_3),$$

i.e. the amount of substrate finally destroyed is proportional to the initial concentration of enzyme. Since K_m is also small, x can become 0.5 to 0.6 a before there is appreciable divergence from proportionality (Fig. 2).

This analysis confirms that the method described has a satisfactory theoretical basis, although the reactions with respect to substrate decomposition are not of the first order. However, in order to convert the units of this method into those of Kat.f. it still remains necessary to determine the

first-order-reaction velocity constant (substrate decomposition) for each sample.

Fig. 3 (curve B) suggested that provided all the substrate is destroyed at the end of the reaction then its decomposition might be of the first order. Fig. 4 confirms this and shows that the initial-reaction velocity is proportional to the substrate decomposition when this is below 0.014M, i.e. under these conditions the reaction was of the first order.

It is therefore possible from the initial slope of Fig. 4 to calculate the first-order velocity constant for this sample since $V = k_1 S$, where V is the reaction velocity, S is the substrate concentration and k_1 is the first-order-reaction velocity constant.

Euler & Josephson (1927), in order to determine Kat.f., used a reaction-velocity constant k which was a function of common logarithms ($\log_{10}$), whereas in the above formula k_1 is a function of natural logarithms ($\ln$).

Therefore it is necessary to substitute k for k_1 in the above formula, i.e.

$$V = 2.3kS.$$

In order that k of this formula should be equivalent to k of Euler & Josephson's formula [$k = 1/t\{\log_{10}(a/(a-x))\}$], where a is the initial substrate concentration and x the substrate decomposed after t min., it was found that S was not the initial substrate concentration but the mean of the initial concentration and of the concentration at the time of taking the reading. V and S can now be obtained by taking any point on the linear first-order portion of Fig. 4, so that k can be determined.

For example, take the point: 8 μ l. of O_2 /min.; 0.01M- H_2O_2 added substrate. Since 3 ml. of reaction mixture is used, then 8/3 μ l. of O_2 is evolved/min./ml. of reaction mixture, i.e. $V = 2.67$, and the initial substrate concentration is 0.01/3M- H_2O_2 , i.e. 37.3 μ l. of available O_2 /ml., and at the time of taking the reading the substrate concentration has become (37.3-8) μ l. of available O_2 /ml. Therefore $S = \frac{1}{2}[37.3 + (37.3 - 8)] = 33.3$ μ l. of available O_2 /ml. Therefore

$$2.67 = 2.3k \cdot 33.3, \text{ by substituting for } V \text{ and } S,$$

$$\text{therefore } k = 0.035 \text{ min.}^{-1}$$

Euler & Josephson's formula for obtaining the Kat.f. value for this method becomes

$$\text{Kat.f.} = k/(\text{g. of protein} \times 50/3)$$

since 3 ml. of reaction mixture was used instead of 50 ml. With this sample of blood, the ratio of haemoglobin to total blood protein was 14.6:17.4, so that a dilution of 5 μ g. of haemoglobin/ml. meant that there was 11.9 μ g. of total blood protein present in the reaction mixture. With these data, it was found that this blood sample had an activity of

Table 1. *Determination of the factor for converting the units of activity of whole blood, obtained by the method described, into Kat.f. units of washed red-blood cells*

k is calculated by applying the formula $V = 2.3kS$ to the results obtained after adding 1 ml. of 0.01 N - H_2O_2 solution to 2 ml. of buffer solution containing 10 μ g. of haemoglobin, the reaction being stopped after 3 min. with 2 ml. of 2 N - H_2SO_4 and the amount of substrate destroyed being measured (see text for detailed example.) The reactions are of the first order under these conditions (Fig. 4). The determination of Kat.f. for washed red cells is described in the text. The mean of the results in the last column is the factor for converting the units obtained by this method, described for whole blood, into Kat.f. units for washed red cells. This factor is 1.37.

Sample	Units of catalase activity		k (min. ⁻¹)	Kat.f. (washed red cells)	Kat.f. (washed red cells) Units of activity (whole blood)
	Whole blood	Washed red cells			
1	160	160	0.035	210	1.31
2	156	159	0.037	222	1.42
3	188	180	0.043	258	1.37
4	170	171	0.040	240	1.41
5	166	165	0.038	228	1.37
6	201	200	0.046	276	1.37
7	144	140	0.032	192	1.34
8	183	186	0.042	252	1.38
9	191	196	0.044	264	1.38

176 Kat.f. units, a figure agreeing well with that of 166 found by Herbert & Pinsent (1948) for their sample.

Thus if the whole-blood protein is estimated it is possible to determine Kat.f. values for whole blood by this method. It was found, however, that with eight different blood samples the activities obtained by this method were the same whether whole blood was used or whether the red cells of these samples were washed free of plasma with physiological saline (0.89 % NaCl) before assay (Table 1). It was also found that washed red cells contained less than 1 % of protein which was not haemoglobin. It is therefore possible to dispense with whole-blood protein determinations and to convert the units of this method obtained for whole blood into Kat.f. values for washed red-blood cells, as follows:

$$\text{Kat.f. (washed red cells)} = k_{(\text{whole blood})} / (10 \times 10^{-6} \times 50/3).$$

For sample 1 (Table 1),

$$\text{Kat.f. (washed cells)} = 0.035 / (10 \times 10^{-6} \times 50/3) = 210.$$

Table 1 shows that the factor for converting the units obtained for whole blood with this method into Kat.f. values for washed red cells is 1.37.

Effect of protein on measurement of catalase. Two experiments were devised to refute the possibility that the protein content of the whole-blood lysate protected the catalase against the destructive effect of hydrogen peroxide. First, a series of readings was made with identical blood samples containing 10 μ g. of haemoglobin/ml. and to which had been added 1 ml. of a solution of crystalline bovine albumin so that the final quantity of 2 ml. of enzyme solution contained 0, 10, 20 and 30 μ g. of albumin. The results obtained are shown in Table 2.

Table 2. *Effect of added bovine crystalline albumin on catalase activity*

Enzyme sample in 2 ml. of buffer	Activity (units)
10 μ g. of haemoglobin	195
10 μ g. of haemoglobin + 10 μ g. of albumin	189
10 μ g. of haemoglobin + 20 μ g. of albumin	187
10 μ g. of haemoglobin + 30 μ g. of albumin	191
30 μ g. of albumin	Nil

Secondly, a nearly pure sample of human red cell, haemoglobin-free catalase (Kat.f. = 23 000) was prepared by the method of Herbert & Pinsent (1948), and this was suitably diluted to give a concentration of about two-thirds of that usually found with blood samples by this method. Three different samples of blood were obtained and their activities determined. These were then diluted to 10 μ g. of haemoglobin/ml. and to 1 ml. of each sample was added 1 ml. of the catalase solution. These results are shown in Table 3.

It follows that no protective action by the protein was found and furthermore that complete recovery of added catalase was obtained.

Catalase content of blood from normal and diseased subjects. This method was applied to blood samples collected from normal people, and from patients of all ages, including infants, whose blood was being investigated during routine clinical laboratory work for a wide variety of conditions, including pernicious anaemia, iron-deficiency anaemia and varieties of anaemia of secondary origin.

It is unfortunate that during the time these investigations were being conducted, no cases of anaemia associated with porphyria, or other unusual examples of altered haemoglobin metabolism, were encountered since it is in these sorts of

Table 3. *Effect of adding catalase to blood samples*

Enzyme sample	Activity (units)	Added activity (units)
Diluted catalase in 1 ml. of buffer	66	—
10 μ g. of haemoglobin (sample 1) in 2 ml. of buffer	141	—
10 μ g. of haemoglobin (sample 1) in 1 ml. of buffer + 1 ml. of diluted catalase	205	64
10 μ g. of haemoglobin (sample 2) in 2 ml. of buffer	165	—
10 μ g. of haemoglobin (sample 2) in 1 ml. of buffer + 1 ml. of diluted catalase	226	61
10 μ g. of haemoglobin (sample 3) in 2 ml. of buffer	196	—
10 μ g. of haemoglobin (sample 3) in 1 ml. of buffer + 1 ml. of diluted catalase	259	63

Table 4. *Results obtained with 145 subjects*

Figures represent units of activity; one observation was made per subject. 94 % of the normal subjects and 90 % of the ill subjects fell within their respective mean $\pm$ S.D. limits.

	No. of subjects	Range	Mean	Standard deviation
Normal subjects	47	75-216	175	38
Ill subjects	98	106-276	184	34

Fig. 5. A scatter diagram constructed from the results obtained during the investigation. Here the units of activity are expressed per constant volume of blood (2 ml. of 1:29 600 dilution) irrespective of the haemoglobin concentration of each sample. A concentration of 14.8 g. of haemoglobin/100 ml. of whole blood was taken as 100 %. (2 ml. of 1:29 600 dilutions of 100 % haemoglobin blood sample contains 10 μ g. of haemoglobins.)

conditions that abnormal results might be found. The results are summarized in Table 4. No correlation was found between the catalase activity and sex or age of the persons examined.

DISCUSSION

In the method described, all the results are expressed as arbitrary units of activity relative to a constant quantity of haemoglobin and irrespective of the actual haemoglobin content of the whole-blood samples. In order to compare the activity of different blood samples, some quantity had to be common to all. Apart from the constant quantity

of haemoglobin, other reference points could have been either a constant blood dilution, as used in most biochemical methods, or a constant number of red-blood cells as used by Richardson, Huddleson & Bethea (1953).

The results obtained and summarized in Table 4 show a narrow distribution, suggesting a fairly close and constant relationship between haemoglobin and catalase which is not disturbed when there is anaemia of any of the usual types, no matter how severe its degree. In order to verify this impression a scatter diagram (Fig. 5) was constructed. In this figure, the abscissa is the catalase activity of each sample expressed as units of activity/2 ml. of a 1:29 600 dilution of whole blood. Since the haemoglobin concentration of each sample was known, and since within the limits of the dilutions used in this method the units of activity of any sample was directly proportional to the haemoglobin content of that sample (see Fig. 2), it was a simple matter to derive the figures plotted from the results obtained and summarized in Table 4.

This graph shows the close linear relationship that exists between catalase activity and the haemoglobin concentration of human blood. This linear relationship suggested that the method used was in fact measuring haemoglobin and not catalase. However, Perlmann & Lipmann (1947) were unable to detect any catalase activity with crystalline haemoglobin, and a report has been published of a family in which several members showed no catalase activity in their blood despite normal haemoglobin concentrations (Takahara, 1952). Furthermore, complete recovery of haemoglobin-free catalase added to the blood samples was obtained by this method (see Table 3).

The use of a constant haemoglobin content, rather than a constant blood dilution, to compare the activity of different samples is supported by the findings shown in Fig. 5. It also justified the decision not to express the activity as a relationship of a fixed number of red cells, since their haemoglobin concentration varies widely in the different types of anaemia. Richardson, Huddleson, Bethea

& Trustdorf (1953) found that the 'index of catalase activity', referred to a constant number of red cells of cattle blood, increased steadily with age of the cattle, whereas the activity expressed/ml. of blood did not change. They also found that the red-cell count decreased with age. Their findings further support the decision to refer the activities of different blood samples to a constant haemoglobin amount, rather than to a constant number of red cells.

It would appear that these two haematin-containing compounds, besides being produced at the same site and contained in the same cell, react similarly to stresses placed upon the bone marrow and always maintain a constant proportion to each other. This similarity of response to marrow disturbance produced by various pathological processes would suggest that catalase and haemoglobin are manufactured in the marrow along a common pathway until the haematin moiety is completed, and that they then follow a different metabolic route for the addition of the globin portion to complete the molecule. Nevertheless, the two final routes must be governed, or govern each other, in some way so that the constant quantitative relationship is maintained.

SUMMARY

1. A simple titrimetric method, giving reproducible results, is described for estimating the catalase activity of blood. The results are expressed in arbitrary units which can readily be converted into the conventional Kat.f. units.

2. The principle of this method depends upon the destructive effect which hydrogen peroxide has upon the enzyme, and the quantities are arranged so that the catalase is destroyed before all the substrate has been decomposed. The reaction may therefore be allowed to proceed to completion and the time factor is no longer critical when making the actual measurements. Under these conditions, there is a linear relationship between the amount of substrate finally utilized and the quantity of enzyme present.

3. There is a constant relationship between catalase activity and haemoglobin concentration, which is not affected by anaemia, age or sex.

4. It is suggested that this relationship indicates a common metabolic pathway for the formation of haemoglobin and catalase, so far as the haematin part of the molecule is concerned.

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