which, although not biologically active, still has the same absorption spectrum as that of non-autoxidizable cytochrome *c*. By extraction with hot 0.1N-acetic acid, Goddard (1944) obtained cytochrome *c* from wheat germ and found that it was less active than the pigment isolated from animal tissues. It is reasonable to assume that as a result of the drastic treatment his preparation contained a considerable amount of autoxidizable cytochrome *c* which would account for the low activity. It is also possible that the apparently high iron content of cytochrome *c* preparations obtained by Tint & Reiss (1950) was not due, as they believed, to any species differences but rather to the presence of an autoxidizable fraction.

The intensity of absorption at 550 m μ . of ferro-cytochrome *c* has commonly been used as a measure of its concentration. However, the presence of an inactive autoxidizable fraction cannot be distinguished by this method. Although the amount of this autoxidizable material is usually negligible it can sometimes lead to a high iron content and thus create a false impression of the purity of the preparation. As a precaution it is therefore recommended, for estimation of cytochrome *c* concentrations by the spectrophotometric method, that a further set of readings be taken after a current of carbon monoxide has been bubbled through the

solution. In this way not only the presence but also a rough estimation of the amount of autoxidizable cytochrome *c* present can be obtained.

SUMMARY

1. Digestion of cytochrome *c* by pepsin produced an autoxidizable pigment. During the digestion the total amount of cytochrome present, autoxidizable and non-autoxidizable, showed a rapid initial drop and a slow subsequent rise.

2. Digestion with papain in absence of an activator raised the iron content of cytochrome *c* from 0.34 to 0.43 % without affecting its catalytic activity. In presence of an activator papain produced an autoxidizable pigment.

3. Digestion with trypsin produced an autoxidizable pigment while chymotrypsin produced a mixture of autoxidizable and non-autoxidizable pigments. The non-autoxidizable part of this mixture, when isolated, contained 0.34 % iron.

4. None of the autoxidizable pigments was active in the succinic oxidase system or in the cytochrome oxidase system with *p*-phenylenediamine as substrate.

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Cytochrome *c* Modified by Digestion with Proteolytic Enzymes

2. PROPERTIES OF PEPSIN-MODIFIED CYTOCHROME *c*

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In the preceding communication (Tsou, 1951) the digestion of cytochrome *c* by proteolytic enzymes and the isolation of autoxidizable fractions of cytochrome *c* thus modified have been described. These autoxidizable cytochrome *c* fractions have been shown to possess several properties in common, namely they are all autoxi-

dizable, they combine with carbon monoxide in the ferrous state and they are not active in biological systems. The properties of one of them, that obtained by digestion with pepsin, will now be examined in greater detail. Some of the findings have already been reported briefly elsewhere (Tsou, 1949).

EXPERIMENTAL

Pepsin-modified cytochrome c (PMc). This was prepared as described (Tsou, 1951) and resisted further attempts at fractionation. The N and Fe contents of both the precipitate and supernatant were found to be unchanged (a) after part of the PMc had been precipitated at pH 6.5, and (b) after precipitating part of the protein at 50% saturation with $(\text{NH}_4)_2\text{SO}_4$.

Table 1. *The iron, total nitrogen, total amino nitrogen, and α -amino nitrogen contents of PMc and ordinary cytochrome c (Fe, 0.43%)*

	Percentage contents of		No. of atoms/atom Fe	
	PMc	c, 0.43% Fe	PMc	c, 0.43% Fe
Iron	2.21	0.43	1	1
Total N	14.72	15.37	26.2	143
Total amino N	3.02	—	5.4	31-32
Total α -amino N	1.89	—	3.4	9-10

Catalase. This was prepared according to the method of Keilin & Hartree (1937); the solution contained about 1% protein.

Ascorbic acid. Solution of desired strength was made up from ascorbic acid and neutralized with *N*-NaOH immediately before use.

Potassium cyanide. Solutions of desired strength were made up from A.R. KCN and neutralized with 0.3*N*-HCl. These were kept in well stoppered bottles at 0° to minimize loss of HCN and used within a few days.

Methylcarbylamine. This was prepared according to the method of Gautier (1869) as modified by Hartley (1928). To ensure the complete removal of cyanide it was washed with dilute NaOH solution, saturated with NaCl, before the final fractional distillation.

β -Phenylhydroxylamine and nitrosobenzene. These were prepared as described by Gattermann (1943).

Cysteine. A solution of desired strength was prepared from cysteine hydrochloride neutralized immediately before use.

Gas mixtures. Mixtures of the required composition were made using the apparatus described by Hartree & Harpley (1949).

Buffers. The following buffer solutions were used for the pH ranges indicated: pH 3-5.5, phthalate; 6-8, phosphate (Sørensen); 8-10.5, borate.

Manometric experiments. These were carried out in Barcroft differential manometers at 38°. The substrate was added (by displacement of a dangling tube) at zero time after temperature equilibration. Readings were taken every 3 or 5 min. after the addition. Except when otherwise specified, the gas phase was air. In all cases the total liquid volume per manometer flask was 3.3 ml.

Spectrophotometric experiments were carried out in a Beckman spectrophotometer.

Amino N. Determinations made by the micro Van Slyke method (Peters & Van Slyke, 1932).

Paper chromatography. The method was essentially as given by Dent (1948) except that a mixture of *n*-butanol and acetic acid (Partridge, 1948) was used instead of collidine.

RESULTS

Properties of PMc

Analytical results. The contents of iron, total nitrogen, total amino nitrogen and α -amino nitrogen of PMc are listed in Table 1. For comparison, the values given for cytochrome *c* with 0.43% iron by Theorell & Åkeson (1941) are also given.

The molecular weight of PMc, calculated on the basis of its iron content, is about 2500, i.e. about 85% of the protein portion of the starting material had been removed by digestion.

Table 2. *The amino-acids (paper chromatography) present in PMc and in ordinary cytochrome c (Fe, 0.43%)*

Amino-acid	PMc	Ordinary cytochrome c
Alanine	+	+
Arginine	—	+
Aspartic acid	+	+
Cystine	+	+
Glutamic acid	+	+
Glycine	+	+
Histidine	+	+
Leucine	+	+
Lysine	+	+
Methionine	—	+
Phenylalanine	+	+
Proline	+	+
Threonine	+	+
Tryptophan	— (?)	+
Tyrosine	—	+
Valine	+	+

The amino-acids present in PMc and those of cytochrome *c* of iron content 0.43% were identified by the two-dimensional paper-chromatographic method. They are listed in Table 2. Cysteine is partially destroyed by acid hydrolysis, but it did show up on some of the papers. Tryptophan is destroyed by acid hydrolysis and hence cannot be detected by this method. PMc, however, gave a negative Hopkins-Cole test after alkaline hydrolysis indicating the absence of this amino-acid. In addition to tryptophan, tyrosine, arginine and methionine were also absent.

Absorption spectrum. The absorption spectrum of modified ferrocytochrome *c* (hereafter referred to as PMc^{++}) was identical with that of ferrocytochrome *c* in the visible region and in neutral and alkaline solutions. The absorption spectrum of pepsin-modified ferricytochrome *c* (PMc^{+++}) closely resembled that of types III and IV of ordinary ferricytochrome *c* as described by Theorell & Åkeson (1941). The absorption spectrum of PMc^{+++} in neutral solution has been described in a previous communication (Tsou, 1949).

Fig. 1. Absorption spectrum of PMc^{+++} at pH 4.5. 0.1 M-Phthalate buffer; Na_2SO_4 , 5 %.

In acid solutions, with a pH of less than 4.5, the absorption spectrum of PMc^{+++} changed to a four-banded pattern very similar to that of type II of ordinary ferricytochrome *c*, i.e. of the acid methaemoglobin type. It showed a sharp, strong band at 630 $\text{m}\mu$, weak bands at 580 and 535 $\text{m}\mu$, and a strong, broad band centred around 500 $\text{m}\mu$. This is shown in Fig. 1.

Haemochromogen-forming groups. Another interesting property of modified cytochrome *c* is that it contains no groupings able to combine with haem in *N*-sodium hydroxide to give a haemochromogen even after being heated for 10 min. at 95°. The formation of a haemochromogen with haem would be revealed by an extra α -band at 560 $\text{m}\mu$.

Oxidation and reduction. PMc^{++} was readily oxidized by air, potassium ferricyanide and cupric salts. PMc^{+++} was easily reduced by sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) and BAL (2:3-dimercaptopropanol) at room temperature and by ascorbic acid at 38°. It was only slowly reduced by ascorbic acid and cysteine

at room temperature, and not reduced by either the succinic dehydrogenase system or by the dihydrocozymase-diaphorase system.

Reaction with cyanide and methylcarbamylamine. PMc^{+++} combined with cyanide and PMc^{++} with both cyanide and methylcarbamylamine. The absorption spectrum of the PMc^{+++} -cyanide compound (shown in Fig. 2) is the same as that of ferricytochrome *c*-cyanide compound as described by Potter (1941) and Horecker & Kornberg (1946). It showed a broad band with its maximum at 537 $\text{m}\mu$. Unlike the reaction between ferricytochrome *c* and cyanide, PMc^{+++} reacted with cyanide instantaneously.

Fig. 2. Absorption spectrum of the PMc^{+++} -cyanide compound (A) as compared with that of PMc^{+++} (B). 0.1 M-Phosphate, pH 7.3; cyanide concentration, 5 mM.

The absorption spectra of the derivatives of PMc^{++} differed from that of PMc^{++} itself most markedly in the relative strength of the α - and β -bands. The absorption spectrum of the cyanide derivative has been given previously (Tsou, 1949), while that of the carbamylamine derivative is shown in Fig. 3. The cyanide compound of PMc^{+++} was reduced with greater difficulty than PMc^{++} itself and the reduction of PMc^{+++} by BAL was found to be slowed down about ten times by 0.003 M-potassium cyanide. Similar results were also obtained with ascorbic acid as reducing agent.

Reaction with carbon monoxide. PMc^{++} combined with carbon monoxide showing two diffuse bands at 530 and 560 $\text{m}\mu$, i.e. about 10 $\text{m}\mu$. towards the shorter wavelength than the corresponding compound of haemoglobin. The absorption spectrum of this compound has been shown in a previous communication (Tsou, 1949). The carbon monoxide derivative of PMc^{++} showed slight light sensitivity

which could be demonstrated in the following way.

A solution of PMc in phosphate buffer, pH 7.3, was reduced by a little solid sodium dithionite and a current of CO was passed through. A strong solution of neutralized KCN was then added. During illumination for several minutes in a strong beam of light, the spectrum of the cyanide compound of PMc^{++} gradually replaced that of the CO compound. As soon as the light was turned off, the spectrum rapidly changed back to that of the CO compound.

Fig. 3. Absorption spectra of the methylcarbylamine (A) and nitrosobenzene (B) compounds of PMc^{++} compared with that of PMc^{++} (C). 0.1 M-Phosphate, pH 7.3; methylcarbylamine, 5 mm; nitrosobenzene, 0.8 mm.

The carbon monoxide compound of PMc^{++} was oxidized by air, but less readily than the pigment itself.

Reaction with nitrosobenzene. PMc^{++} combined with nitrosobenzene forming a compound with an absorption spectrum closely similar to that of the carbon monoxide compound (Fig. 3).

Reaction with nitric oxide. PMc in both the oxidized and the reduced state combined with nitric oxide. The compound with PMc^{+++} had an absorption spectrum closely similar to that of the ferri-cytochrome c-NO compound, while the absorption spectrum of $\text{PMc}^{++}\text{-NO}$ is of the type of the carbon monoxide and nitrosobenzene compounds. A comparison of the properties of PMc with those of ordinary cytochrome c at neutral and alkaline pH (types III and V) and with haemoglobin is given in Table 3.

Catalytic activity of PMc

On shaking a few drops of a solution of hydrogen peroxide with a PMc solution, a rapid evolution of oxygen was observed and the colour of the solution was rapidly changed to a light brown on account of destruction of the pigment. Cyanide, at a concentration of 0.002M, strongly inhibited this decomposition. The Kat.f. (Euler & Josephson, 1927) of PMc was about 400 (Keilin & Hartree, 1937). PMc showed no peroxidatic activity at all with pyrogallol as substrate.

PMc was inactive in both the succinic oxidase system and the cytochrome oxidase system with *p*-phenylenediamine as substrate (Tsou, 1951). When tested in the cytochrome oxidase system using ascorbic acid as a secondary substrate, PMc showed, surprisingly, a pronounced activity. As PMc is rapidly oxidized by air, the presence of cytochrome oxidase was found to be quite unnecessary. In fact, in the catalysed oxidation of ascorbic acid, PMc acted not as an essential link in the cytochrome oxidase system but as an artificial ascorbic acid oxidase. This is clearly shown in Fig. 4. This oxidation of ascorbic acid appeared to be the most pronounced catalytic property of PMc, therefore it will be examined in greater detail.

Effect of 2,2'-dipyridyl on the reaction rate. The following experiments show that the catalytic effect of PMc on the rate of oxidation of ascorbic acid was due to the PMc itself and not to any free iron that might be present in, or produced from, PMc by destructive agents (e.g. hydrogen peroxide from autoxidation of ascorbic acid).

Table 3. Comparison of the properties of PMc with those of ordinary cytochrome c in neutral (c-III) and alkaline (c-V) solutions and haemoglobin (Hb)

	c-III	c-V	PMc	Hb
Autoxidizability	-	+	+	-
Reactivity of ferrous form with CO	-	+	+	+
Light sensitivity of CO compound	.	+	+	+
Reactivity of ferric form with cyanide	+	+	+	+
Reactivity of ferrous form with cyanide	-	+	+	-
Reactivity of ferrous form with carbylamine	-	+	+	-
Reactivity of ferrous form with nitrosobenzene	-	-	+	+
Reactivity of ferric form with nitric oxide	+	.	+	+
Reactivity of ferrous form with nitric oxide	-	.	+	+
Reaction with hydrogen peroxide	-	Destroyed with evolution of O_2	-	Oxidized
Haemochromogen-forming groups	+	+	-	+

In presence of ascorbic acid any free iron present would be in the ferrous state and would combine with 2:2'-dipyridyl to form a stable complex. It will be noted that saturation with 2:2'-dipyridyl did not affect the initial rate of oxygen uptake (Fig. 5) but that the rate fell off more rapidly in presence of 2:2'-dipyridyl. It will be shown later that hydrogen peroxide is produced during this oxidation of

Fig. 4. Catalysed oxidation of ascorbic acid by PMc. 0.065 M-phosphate, pH 7.3; 0.025 M-ascorbic acid; temp., 38°. Curves A and B show the oxidation of ascorbic acid by PMc, 9.4 μ M, in the presence and absence of a heart-muscle preparation (0.58 mg. fat-free dry weight) respectively. Curves C and E show the oxidation with ordinary cytochrome *c*, 9.4 μ M, in the presence and absence of the same amount of heart-muscle preparation respectively. Curve D shows the autooxidation of ascorbic acid.

ascorbic acid, and it is therefore reasonable to conclude that the initial oxidation of ascorbic acid was due to PMc alone, whereas in the later stages of the reaction the iron set free from PMc by the action of hydrogen peroxide also contributed. By removing the ferrous ion as soon as it was formed, 2:2'-dipyridyl reduced the rate of oxidation of ascorbic acid in the later part of the reaction.

Coupled oxidation with catalase and ethanol. During the catalysed oxidation of ascorbic acid oxygen is reduced to hydrogen peroxide which can be demonstrated in a coupled oxidation with catalase and ethanol. This is shown in Fig. 6. It will be seen that the catalase partly protected the PMc from destruction by hydrogen peroxide so that the rate of oxygen uptake remained steadier in presence of catalase than in its absence. The addition of ethanol

Fig. 5. Effect of 2:2'-dipyridyl on the PMc-catalysed oxidation of ascorbic acid. Conditions as in Fig. 4. Curves A and B show the oxidation catalysed by 7 μ M-PMc in the absence and presence of 2:2'-dipyridyl respectively. Curve C shows control, autooxidation of ascorbic acid.

Fig. 6. Coupled oxidation with catalase and ethanol. Conditions as in Fig. 4, PMc, 9.4 μ M. Curve A, PMc catalysed oxidation of ascorbic acid; curve B, as A in presence of ethanol; curve C, as A in presence of catalase; curve D, as A in presence of both catalase and ethanol.

alone, as was to be expected, produced no effect at all while the presence of both ethanol and catalase resulted in an increased rate of oxygen uptake due, no doubt, to utilization of hydrogen peroxide in the oxidation of ethanol. In this reaction ascorbic acid was oxidized far beyond the dehydroascorbic acid stage. In fact, about three to four times the theoretical oxygen uptake for the formation of dehydroascorbic acid was obtained and carbon dioxide was also produced.

Effect of pH on the reaction rate. The effect of pH on both the autoxidation and the PMc-catalysed oxidation of ascorbic acid is shown in Fig. 7. It must be pointed out here that the oxygen uptake obtained at alkaline pH was somewhat less certain owing to the rapid production of carbon dioxide which upset the pH of the buffered medium.

Fig. 7. Effect of pH on the PMc-catalysed oxidation of ascorbic acid. Conditions as in Fig. 4, PMc, 7 μ M. Buffers: 0.1 M-phthalate, pH 3-6; 0.085 M-phosphate, pH 6-8; 0.1 M-borate, pH 8-10. Curve A, PMc-catalysed oxidation of ascorbic acid; curve B, blank showing autoxidation of same; curve C, PMc-catalysed oxidation of same corrected for blank.

Effect of PMc concentration on reaction rate. That the rate of oxidation of ascorbic acid is directly proportional to the amount of PMc present is shown in Table 4. The average value of Q_{O_2} (μ l. oxygen uptake/mg. PMc/hr.) at 38° and pH 7.3 was 11,560.

Effect of ascorbic acid concentration on the reaction rate. This is shown in Fig. 8. It will be noted that the ascorbic acid concentration used in obtaining the Q_{O_2} (Table 4) was still far below the optimum.

Inhibition by carbon monoxide. It has been shown above that the reduction of PMc by ascorbic acid is

somewhat accelerated in the presence of carbon monoxide. However, the reoxidation of the PMc-carbon monoxide compound by air is much more difficult than that of PMc itself. Manometric

Table 4. Effect of PMc concentration on the rate of oxidation of ascorbic acid at 38°

(Experiments carried out in Barcroft differential manometers. Each right-hand Barcroft flask contained 0.065 M-phosphate, pH 7.3; ascorbic acid, 0.025 M; 0.1 ml. of tenfold diluted catalase; and the concentration of PMc as specified in the table. KOH papers were used in the centre well. Q_{O_2} was calculated from the O_2 uptake in the first 10 min. and expressed as μ l. O_2 uptake/mg. dry wt. of PMc/hr.)

PMc (μ M)	O_2 uptake in first 10 min. (μ l.)	O_2 uptake in 1st 10 min. corrected for blank (μ l.)	Q_{O_2}
Nil	18	—	—
2.3	55	37	12,400
4.7	88	70	11,600
7.0	120	102	11,400
9.4	155	137	11,500
14.1	212	194	10,900
		Mean	11,560

Fig. 8. Effect of ascorbic acid concentration on the PMc-catalysed oxidation of ascorbic acid. PMc, 7 μ M; buffer and temperature as in Fig. 4.

measurements showed that carbon monoxide inhibited the catalysed oxidation of ascorbic acid by PMc to a considerable extent, e.g. in a mixture of 20% oxygen and 80% carbon monoxide, the reaction was inhibited by 85%. In agreement with the spectroscopic observations, the carbon monoxide inhibition was found to be somewhat light sensitive (Fig. 9).

The inhibition at different ratios of $CO:O_2$, but with a constant partial pressure of oxygen, was also estimated. The partition constant between oxygen and carbon monoxide of PMc was then calculated (Table 5). The calculations were made on the assumption that the carbon monoxide complex was completely inactive in the oxidation of ascorbic acid.

Fig. 9. Light dissociation of the $\text{PMc}^{++}\cdot\text{CO}$ complex. Conditions as in Fig. 4, PMc, $7\mu\text{M}$. Curve C, control in air; curves A, A', B and B', in 80% CO and 20% O_2 . Curve A, reaction in light throughout; curve A', reaction in light from start to 12th min., in dark from 12th to 27th min.; curve B, in dark throughout; curve B', in dark from start to 12th min. and in light from 12th to 27th min.

Table 5. The partition constant of PMc between CO and O_2

(Experimental conditions as in Table 4. PMc concentration, $8.4\mu\text{M}$. Partition constant calculated as

$$k = \frac{[\text{PMc}^{++} - \text{CO}]}{[\text{PMc}^{++}]} \frac{p_{\text{O}_2}}{p_{\text{CO}}} = \frac{I \cdot R}{100 - I},$$

where $[\text{PMc}^{++}]$ and $[\text{PMc}^{++} - \text{CO}]$ are the molar concentrations of PMc and its CO compound respectively; p_{O_2} and p_{CO} are the partial pressures of O_2 and CO respectively; I is the % inhibition and R the ratio of O_2 :CO.)

Composition of gas phase (%):

O_2	20	20	20	20
N_2	80	70	50	0
CO	0	10	30	80
O_2 uptake first 10 min. ($\mu\text{l.}$), corrected for blank	113	72.6	39	17.5
Inhibition (I) (%)	—	35.8	65.5	84.5
Ratio, O_2 :CO (R)	—	2	0.67	0.25
Partition constant	—	1.11	1.21	1.36

Table 6. Inhibition of the PMc-catalysed oxidation of ascorbic acid

(Conditions as in Table 4. PMc concentration, $9.4 \times 10^{-6}\text{M}$. Temp. 38° .)

Inhibitor	Concentration (mM)	Inhibition (%)
Cyanide	0.8	57
	1.6	74
	3.2	87
Methylcarbylamine	1.5	46
	3.0	65
	6.0	93
Sodium nitrite	1.3	90
	2.6	100
β -Phenylhydroxylamine	0.4	48
	0.8	74

Other inhibitors. Cyanide, methylcarbylamine, sodium nitrite and β -phenylhydroxylamine also inhibited this oxidation of ascorbic acid. A summary of the results is given in Table 6.

18 mM-Sodium azide, 18 mM-pyrophosphate and 0.8 mM-phenylthiourea had no effect on this reaction.

PMc also strongly catalysed the oxidation of dihydroxymaleic acid. It was ineffective in the oxidation of either cysteine or *p*-phenylenediamine.

DISCUSSION

The fact that the pepsin-modified cytochrome *c* has an absorption spectrum closely similar to that of ordinary cytochrome *c* seems to indicate that both the prosthetic group and that part of the cytochrome *c* protein which is in the immediate vicinity of the prosthetic group has not been appreciably changed during the digestion. The presence of cystine and histidine in PMc also supports this view if Theorell's (1941) hypothesis of the linkage of the prosthetic group to the protein is accepted. Chemical analysis points to the molecule of PMc consisting of three or four peptide chains containing approximately fifteen amino-acids. However, the chemical structure of the relatively small molecule of PMc, which may not be too difficult to elucidate, does not necessarily represent that part of the original cytochrome mole-

cule which was in the immediate vicinity of the prosthetic group. The earlier claim of Wasteneys & Borsook (1924, 1925) that pepsin has synthetic as well as hydrolytic action does not seem to have been substantiated by later workers (Folley, 1932; Butler, Dodds, Phillips & Stephen, 1948), but nevertheless the possibility cannot yet be ruled out. The changes in the total concentration of cytochrome *c* during the pepsin digestion (cf. Tsou, 1951) give definite indication that some rearrangement within the molecule is taking place.

Most haematin compounds possess some oxidative, catalytic as well as peroxidatic, activity. As a haematin with a limited number of amino-acids attached, PMc is remarkable in that although

it has a high oxidative activity, it is very low in catalytic activity and is devoid of peroxidatic activity.

SUMMARY

1. The iron, total nitrogen, total amino-nitrogen and α -amino-nitrogen contents of PMc were determined and the amino-acids present were identified.

2. The adsorption spectra of the oxidized and reduced forms of this pigment resemble closely those of ordinary cytochrome *c*.

3. This pigment combines with cyanide, methyl-carbarylamine, carbon monoxide, nitric oxide and nitrosobenzene. It is autoxidizable and shows properties similar to type V of ordinary cytochrome *c* (i.e. cytochrome *c* at pH 13) on the one hand and haemoglobin on the other.

4. It is inactive in both the succinic oxidase system and the cytochrome oxidase system with *p*-phenylenediamine as substrate, but strongly catalyses the oxidation of ascorbic acid. As an artificial ascorbic acid oxidase, it has a Q_{10} of 11,500. This catalysed oxidation of ascorbic acid is inhibited by cyanide, methylcarbarylamine, nitrosobenzene and carbon monoxide, the latter inhibition being somewhat light sensitive.

5. The pigment shows little catalytic activity and is devoid of peroxidatic activity.

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The Haemoglobins of the Human Foetus and Infant. Electrophoretic and Spectroscopic Differentiation of Adult and Foetal Types

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The differences between human adult and foetal haemoglobin have been summarized recently by Joep & O'Brien (1949) and by Kendrew (1949). Apart from the difference in the rate of denaturation by alkali, these differences were not sufficiently great to allow the determination of the small amounts of adult haemoglobin in the haemoglobin of the human foetus during early development and no such attempt has been reported. The statements on the relative amounts of the two types of haemoglobin at and after birth are not in good agreement. Most of the evidence is based on the difference in the resistance to denaturation by alkali, the foetal

haemoglobin being the more resistant type. According to Haurowitz (1929, 1930) the blood of the newborn contains 80% resistant and 20% non-resistant haemoglobin. Jonxis (1949), at variance with a previous statement (Jonxis, 1948), reports that at birth the percentage of 'later' haemoglobin in healthy newborn infants ranges between 2 and 25% and that after 22 weeks all foetal haemoglobin disappears. Brinkman & Jonxis (1935) find that the foetal haemoglobin disappears entirely at 7 months after birth. The two types of haemoglobin in the newborn are immunologically different (Darrow, Nowakovsky & Austin, 1940). In the haemoglobin