SUMMARY

1. The reactions between chymotrypsin and the *p*-nitrophenyl ester of *N*-benzyloxycarbonyl L-tyrosine have been studied by a fast-reaction photometric method.

2. It has been shown that an *N*-benzyloxycarbonyl-L-tyrosine–chymotrypsin compound is formed as an intermediate in the enzymic hydrolysis of this substrate. The formation of this intermediate compound is so fast ($k > 1000$ sec.$^{-1}$) that its kinetics could not be studied.

3. A comparison of the reaction parameters of the chymotrypsin-catalysed hydrolysis of the amide, ethyl ester and *p*-nitrophenyl ester of tyrosine gives further support to a three-step reaction scheme for enzymic hydrolysis, which has been previously proposed.

We are most grateful to Dr C. J. Martin for a sample of the *p*-nitrophenyl ester of *N*-benzyloxycarbonyl-L-tyrosine as well as for some helpful advice.

REFERENCES

Bender, M. L., Ginger, R. D. & Kemp, K. C. (1954). *J. Amer. chem. Soc.* **76**, 3350.

Bender, M. L. & Turnquest, B. W. (1955). *J. Amer. chem. Soc.* **77**, 4271.

Bender, M. L. & Turnquest, B. W. (1957*a*). *J. Amer. chem. Soc.* **79**, 1652.

Bender, M. L. & Turnquest, B. W. (1957*b*). *J. Amer. chem. Soc.* **79**, 1656.

Bernhard, S. A. & Gutfreund, H. (1958). *Proc. int. Symp. Enzyme Chem., Tokyo*, p. 124.

Biggs, A. I. (1954). *Trans. Faraday Soc.* **50**, 800.

Dixon, G. H. & Neurath, H. (1957). *J. Amer. chem. Soc.* **79**, 4558.

Foster, R. J. & Niemann, C. (1955). *J. Amer. chem. Soc.* **77**, 1886.

Gibson, Q. H. (1954). *Disc. Faraday Soc.* **17**, 137.

Gutfreund, H. (1955). *Disc. Faraday Soc.* **20**, 167.

Gutfreund, H. & Sturtevant, J. M. (1956*a*). *Biochem. J.* **63**, 656.

Gutfreund, H. & Sturtevant, J. M. (1956*b*). *Proc. nat. Acad. Sci., Wash.*, **42**, 719.

Hammond, B. R. & Gutfreund, H. (1955). *Biochem. J.* **61**, 181.

Hartley, B. S. & Kilby, B. A. (1954). *Biochem. J.* **56**, 288.

Jandorf, B. J. & Michel, H. O. (1957). *Annu. Rev. Biochem.* **21**, 351.

MacAllister, R. V. (1949). Ph.D. Thesis: California Inst. of Tech., Pasadena.

Martin, C. J., Golubow, J. & Axelrod, A. E. (1959). *J. biol. Chem.* **234**, 294.

Northrop, J. H., Kunitz, M. & Herriott, R. M. (1948). *Crystalline Enzymes*, 2nd ed. New York: Columbia University Press.

Snoke, J. E. & Neurath, H. (1949). *Arch. Biochem.* **21**, 351.

Spencer, T. & Sturtevant, J. M. (1959). *J. Amer. chem. Soc.* **81**, 1874.

Oxidation of Serotonin and 5-Hydroxyindoles during the Denaturation of Oxyhaemoglobin

BY J. J. BLUM* AND N.-S. LING

Mental Health Research Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan, U.S.A.

(*Received* 22 *December* 1958)

Stacey (1956) reported that serotonin (5-hydroxytryptamine) disappears when it is incubated with washed erythrocytes. In a study of the transport of serotonin into erythrocytes we found no serotonin inside the cells, and it seemed possible that the serotonin was being destroyed by a factor in the erythrocytes. This paper explains why serotonin appeared to be destroyed by erythrocytes. A preliminary report of this work has already appeared (Ling & Blum, 1958).

EXPERIMENTAL AND RESULTS

Blood (about 15 ml.) was obtained from two rats lightly anaesthetized with ether which were bled from the neck into a polyethylene beaker containing 10 ml. of 0·85% NaCl with either 0·1 g. of ethylenediaminetetra-acetic acid or 6 mg. of heparin. The erythrocytes were then centrifuged for 3 min. at 2000 *g* and the supernatant fluid was decanted. The cells were resuspended in 47–50 ml. of 0·12 M-NaCl–0·04 M-Na_2HPO_4 buffer, pH 7·4. The cells were washed three times in about 40 ml. of this buffer. Blood obtained from other species was treated in the same way. When necessary, the washed erythrocytes were haemolysed by adding 2–5 vol. of water. Crystalline haemoglobin was prepared from dog blood by the procedure of Drabkin (1946).

The creatinine sulphate salt of serotonin, bufotenin (*NN*-dimethylserotonin) mono-oxalate, tryptamine hydrochloride, indol-3-ylacetic acid and 5-hydroxyindol-3-ylacetic acid (5-HIAA) were purchased from the California Biochemical Foundation. Crystalline egg albumin, serum globulin, cytochrome *c*, glutathione and 5-hydroxytryptophan were purchased from the Nutritional Biochemical Corp. Catalase was purchased from Worthington Biochemicals Inc., haemin from Eastman Organic Chemicals

* Present address: Gerontology Branch, Baltimore City Hospitals, Baltimore 24, Maryland, U.S.A.

Dept. and iproniazid (*N-iso*nicotinoyl-*N'-iso*propylhydrazine) from Hoffman-La Roche and Co. All other chemicals were reagent-grade.

Serotonin and other 5-hydroxyindoles were assayed spectrophotofluorimetrically in an Aminco–Bowman spectrophotofluorimeter. Deproteinization of samples to be assayed was accomplished with trichloroacetic acid. A sample (2 ml.) of the deproteinized filtrate was mixed with 1 ml. of 9 N-HCl in a 1 cm. square cuvette and the fluorescence was measured with an exciting wavelength (λ_e) of 300 mμ and a fluorescent wavelength (λ_f) of 555 mμ (Udenfriend, Bogdanski & Weissbach, 1955). Since trichloroacetic acid reduces the fluorescence of some 5-hydroxyindoles, standard solutions contained the same final concentration of trichloroacetic acid. In most of the experiments reported here the extinction at 300 mμ of the solutions whose fluorescence was measured was less than 0·4, a range in which fluorescence increases linearly with increasing serotonin concentration. Serotonin was also assayed colorimetrically by the procedure of Udenfriend, Weissbach & Clark (1955).

Preliminary experiments with washed rat erythrocytes confirmed Stacey's (1956) observation that when serotonin was added to a suspension of these cells the serotonin rapidly disappeared from the external solution. It was then found that serotonin also disappeared from solution when haemolysates were used, and it was noted that the deproteinized filtrates were often pink. Since it was possible that erythrocyte 'ghosts' were the active agents, 'ghost'-free haemolysates were prepared. It was found that the activity was unaffected by the removal of the 'ghosts' and thus the presence of an active protein was indicated. The results shown in Table 1 suggested that the reaction might be non-enzymic, since the amount of serotonin lost did not depend on time (for times greater than about 20 sec.), and since the amount of serotonin lost was not simply related to the amount of oxyhaemoglobin added (Fig. 1, curve *B*). When the haemolysate was heated at 60° for 20 min. there was a gradual loss of the ability to destroy serotonin. Heating for 2–3 min. at 80° caused a brown precipitate to form and resulted in a 70 % loss of serotonin-destroying ability. There was little effect of pH between 4·0 and 10·0 on the ability of a haemolysate to destroy serotonin (Table 2), but at pH 3·0 where a brownish precipitate formed, serotonin-destroying activity was lost. The apparently non-enzymic nature of the reaction and the range of heat and of pH sensitivity suggested that the active protein was haemoglobin.

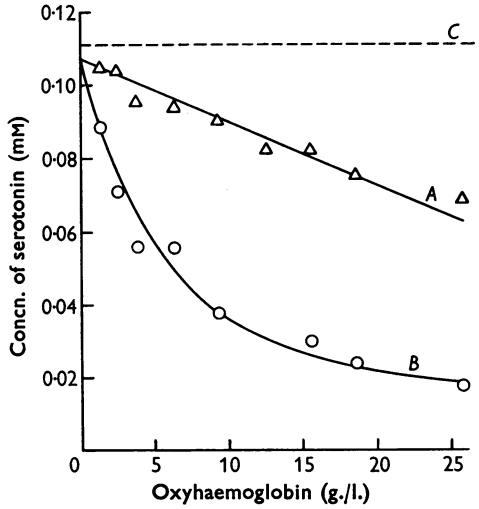

Fig. 1. Destruction of serotonin by oxyhaemoglobin. Curve *B* represents a reaction mixture containing the indicated amounts of crystalline dog oxyhaemoglobin, to which was added serotonin to make a final concentration of 0·11 mM, followed by trichloroacetic acid to give a final concentration of 4·6 % (w/v). Conditions for curve *A* were the same except that the trichloroacetic acid was added to the oxyhaemoglobin before the addition of serotonin. Curve *C* is the serotonin concentration that would have been found if no serotonin had been destroyed. Measurements were made by the colorimetric method of Udenfriend, Weissbach & Clark (1955).

Table 1. *Effect of time of incubation on the reaction of serotonin with different amounts of haemolysed erythrocytes*

Washed erythrocytes from 6·5 ml. of rat blood were suspended in 20 ml. of buffer. This was diluted 1:10 with water to make the haemolysate. To the indicated amounts of haemolysate at room temperatures was added 0·364 μmole of serotonin. Samples were withdrawn at the times shown and were deproteinized with trichloroacetic acid (final concn. 0·306 M) and assayed spectrophotofluorimetrically.

	Serotonin found					
Vol. of haemolysate (ml.)	... 0·5		1·0		3·0	
Time (sec.)	(μmole)	(% loss)	(μmole)	(% loss)	(μmole)	(% loss)
20	0·050	15·0	0·045	23·5	0·032	45·6
100	0·056	4·8	0·048	18·4	0·031	47·2
170	0·055	6·5	0·049	16·7	0·033	43·8
250	0·054	8·2	0·045	23·5	0·033	43·8
700	0·055	6·5	0·049	16·7	0·032	45·6

Table 2. *Effect of* pH *on the reaction of serotonin with haemolysed erythrocytes*

Washed erythrocytes from 15 ml. of rat blood were haemolysed with water to a final volume of 50 ml. To 1 ml. of haemolysate were added 2 ml. of 0·2M-buffer of composition and pH shown in the table, and then 5 ml. of the appropriate concentration of serotonin in water. The resulting mixture was incubated for 5 min. at room temperature, and then 2 ml. of 10% (w/v) trichloroacetic acid was added. The serotonin was then assayed spectrophotofluorimetrically. Appropriate standards and blanks were also prepared. Sodium acetate and sodium tartrate were employed, and the pH was adjusted with NaOH or HCl as necessary. Tris, 2-amino-2-hydroxymethylpropane-1:3-diol.

Serotonin in incubation mixture (μmole)	Serotonin loss (%)				
	Acetate pH 4·0	Acetate pH 6·0	Tris pH 8·0	Tris pH 10·0	Tartrate pH 3·0
0·0036	94	99	99	97	—
0·0073	93	99	99	98	19
0·0127	92	96	99	98	—
0·0182	91	97	97	97	12
0·0364	73	89	90	90	—
0·0910	39	62	65	70	2
0·1820	37	40	48	50	—

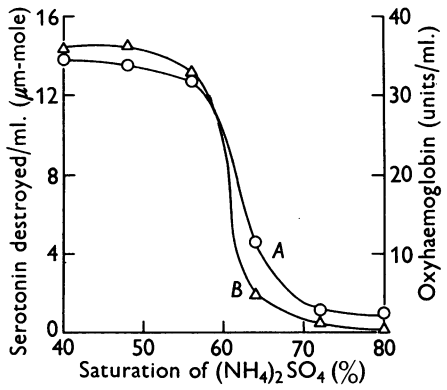

Fig. 2. Ammonium sulphate fractionation of active protein. The washed erythrocytes from 13 ml. of rat blood were haemolysed with water to a final volume of 50 ml. Samples (5 ml.) were then adjusted to 25 ml. by the addition of appropriate amounts of saturated $(NH_4)_2SO_4$ and of water. The tubes were kept at 4° for 3 hr. and then centrifuged for 10 min. at 12 800 *g*. To the supernatant fluid serotonin was added to make the final concentration 14·2 μm-moles/ml. After a few minutes trichloroacetic acid (final concn. 0·3M) was added and serotonin was assayed spectrophotofluorimetrically. Portions of the supernatant fluid were diluted with appropriate amounts of water and their extinction was measured in an Evelyn colorimeter. An oxyhaemoglobin unit is defined as the quantity of oxyhaemoglobin in aqueous solution which has an extinction of unity in this instrument, with a 420 mμ filter. Curve *A* shows the amount of serotonin destroyed, and curve *B* is proportional to the oxyhaemoglobin concentration.

Nature of the active protein

An ammonium sulphate-fractionation procedure was employed to purify the active protein. As is evident from Fig. 2, the serotonin-destroying activity (curve *A*) was precipitated under the same conditions as the oxyhaemoglobin (curve *B*). Since rat oxyhaemoglobin is difficult to redissolve after crystallization, crystalline dog oxyhaemoglobin was tested and found to destroy serotonin (Fig. 3). The results from this and other experiments show that 95% loss of serotonin occurs when the molar ratio of oxyhaemoglobin to serotonin is 5 to 1 or greater, and that 30% loss occurs when the ratio of oxyhaemoglobin to serotonin is 1 to 5 or less. We have been unable to obtain precise stoicheiometric relations for several reasons. Experiments with a fixed amount of oxyhaemoglobin and varying serotonin (Fig. 3) have a rather sharp break, suggesting a stoicheiometric relation between the amount of oxyhaemoglobin and the amount of serotonin which can be destroyed. Experiments with a fixed amount of serotonin and varying amounts of oxyhaemoglobin (Fig. 1, curve *B*), however, do not show a linear relation between the amount of serotonin destroyed and the amount of oxyhaemoglobin present. Furthermore, it is found that at very high concentrations of serotonin

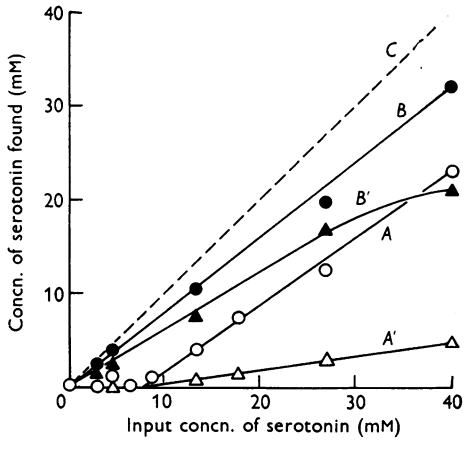

Fig. 3. Destruction of serotonin in the presence of a fixed amount of oxyhaemoglobin. Curves *A* and *A'* (open symbols) represent a reaction mixture containing 12·4 mg. of crystalline dog oxyhaemoglobin/ml., to which was added the indicated amounts of serotonin, followed by trichloroacetic acid to give a final concentration of 4·6% (w/v). Conditions for *B* and *B'* (filled-in symbols) were the same except that the trichloroacetic acid was added to the oxyhaemoglobin before the addition of serotonin. Curve *C* is a reference curve for 100% recovery of added serotonin. ○, ●, Measurements obtained colorimetrically; △, ▲, spectrophotofluorimetric measurements of portions of the same reaction mixtures.

Table 3. *Effects of carbon monoxide on the oxidation of serotonin by oxyhaemoglobin*

The components of the reaction mixture were added in the sequence shown, and the serotonin remaining in the filtrate was assayed spectrophotofluorimetrically. Final volume in each tube was 4·5 ml. The final concentrations were: oxyhaemoglobin (O_2Hb), 18·7 mg./ml.; serotonin (S), 0·111 mM; trichloroacetic acid (TCA), 5·6% (w/v); buffer (B), 0·02M-Na_2HPO_4, pH 8·5; CO was bubbled through the mixture for 2 min.

Tube no.	1	2	3	4	5	6
Sequence of addition of reagents	O_2Hb TCA S CO	O_2Hb S CO TCA	O_2Hb CO S TCA	O_2Hb B CO TCA	O_2Hb CO TCA B	O_2Hb S TCA B
Final concn. of serotonin (mM) ...	0·088	0·079	0·090	0·000	0·000	0·005

Table 4. *Effect of addition of oxygen on the oxidation of serotonin by haemoglobin*

Serotonin (10 μmoles) was added to two solutions, each containing 100 mg. of crystalline dog haemoglobin. The final volume was adjusted to 25 ml. The serotonin was assayed spectrophotofluorimetrically in the presence of 0·113M-trichloroacetic acid.

Treatment	Time (sec.)	Loss (%)
Bubbling oxygen	270	27
	445	27
	910	28
	2000	29
None	230	27
	480	26
	940	29
	2020	27

(greater than 10 mM) the amount lost appears larger when the deproteinized reaction mixture is assayed spectrophotofluorimetrically (Fig. 3, curves *A'* and *B'*) than when the same mixture is assayed colorimetrically (Fig. 3, curves *A* and *B*). The difference between the two methods at high serotonin concentrations may be an artifact resulting from the high extinctions (greater than 2) at 300 mμ of the solution to be assayed in the photofluorimeter. At lower concentrations both methods give results which agree completely. Another complication arises from the observation that the controls, in which oxyhaemoglobin was denatured by trichloroacetic acid before the addition of serotonin, are still capable of destroying some serotonin (Fig. 1, curve *A*), though without the production of the characteristic pink product to be described below.

Exposure of oxyhaemoglobin to CO (Table 3) caused an almost complete loss of activity, thus suggesting that the Fe^{2+} ion of the haem is necessary for the destruction of serotonin. The finding that even large concentrations (0·3M) of KCN do not greatly reduce the serotonin-destroying activity of a given amount of oxyhaemoglobin is further evidence that the active protein is ferrohaemoglobin. Incubation of serotonin with $FeCl_3$ instead of oxyhaemoglobin caused a very slow destruction of serotonin, and hence traces of it could not be responsible for the activity of oxyhaemoglobin. No activity was detected with solutions of crystalline egg albumin (0·19%), bovine serum globulin (0·19%), catalase (0·38%) or cytochrome *c* (0·14%). Haemolysates of human, turkey, pig, cow, chicken, rabbit and cat erythrocytes were as active as rat-erythrocyte haemolysates.

When O_2 was bubbled through a reaction mixture there was no increase in the destruction of serotonin (Table 4), but when O_2 was removed from oxyhaemoglobin (and from the serotonin and trichloroacetic acid solutions) there was little or no loss of serotonin (Table 5). Glutathione and $NaHSO_3$ (0·33M) did not prevent the destruction of serotonin by oxyhaemoglobin. Ascorbic acid, however, largely prevented the destruction of serotonin (Table 6).

No change was found in the light absorption of oxyhaemoglobin after the addition of serotonin. Since it seemed possible that the ability of oxyhaemoglobin to destroy serotonin was due to its well-known pseudo-peroxidase activity (Willstätter & Pollinger, 1923), a solution of crystalline dog oxyhaemoglobin was pre-incubated with 5 mg. of catalase for 10 min. before the addition of serotonin. Upon deproteinizing with trichloroacetic acid and assaying, there was found little or no difference in the pink colour or in the amount of serotonin destroyed as compared with a control not treated with catalase. The possibility that organic peroxides associated with the crystalline oxyhaemoglobin were responsible for the activity was further checked by incubating quinol with the oxyhaemoglobin. There was no colour in the deproteinized filtrate, although the addition of H_2O_2 to a sample of quinol rapidly yielded a yellowish brown.

Carbon monoxide protects serotonin against oxidation by oxyhaemoglobin even when the CO is added after the serotonin and oxyhaemoglobin have been in contact (Table 3). Thus the oxidation of serotonin does not occur until the denaturation of oxyhaemoglobin takes place. The oxidation occurs whether the denaturation is caused by butanol or by trichloroacetic acid. Serotonin is oxidized more easily than ascorbic acid and much more easily than glutathione. Unlike ascorbic acid and glutathione

Table 5. *Effect of haemoglobin and of oxyhaemoglobin on serotonin*

The components were added in the sequence shown. Deoxygenation was achieved by evacuation of the system with an oil pump followed by flushing with N_2 and re-evacuation. The final concentrations were (abbreviations as in Table 3): Hb or O_2Hb, 13·4 mg./ml.; S, 0·111 mM; TCA, 5·6% (w/v). Total volume was 4·5 ml. Serotonin in the filtrate was assayed spectrophotofluorimetrically.

Sequence of addition of reagents	No O_2		O_2	
	Hb S TCA	Hb TCA S	O_2Hb S TCA	O_2Hb TCA S
Final concn. of serotonin (mM) ...	0·096	0·096	0·014	0·100

Table 6. *Effect of ascorbic acid on the reaction of serotonin with haemolysed erythrocytes*

A dialysed haemolysate (3 ml.) of dog erythrocytes, containing 46·8 mg. of oxyhaemoglobin/ml. was placed in each tube, followed by 10 ml. of ascorbic acid of the desired concentration dissolved in 0·2 M-Na_2HPO_4, pH 7·4, and then by 0·5 μmole of serotonin in 1 ml. of water. A portion (2 ml.) of 25% (w/v) trichloroacetic acid was added and serotonin in the filtrate was measured at λ_a 300 mμ, λ_f 360 mμ, without the addition of 3 N-HCl.

Tube no.	Ascorbic acid (mM)	Serotonin (% loss)	Colour
1	7·14	40	None
2	4·28	40	None
3	2·86	40	None
4	1·43	42	Barely pink
5	0·71	50	Light pink
6	0·36	61	
7	0·29	62	
8	0·21	74	
9	0·14	77	Increasing pink
10	0·07	82	
11	0·04	89	
12	0·00	94	

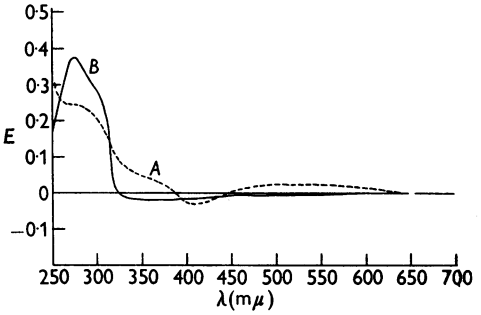

Fig. 4. Absorption of serotonin and of serotonin oxidation product(s). Washed erythrocytes from 14·5 ml. of heparinized rat blood were haemolysed with water to a final volume of 50 ml. Curve *A* is the spectrum of the filtrate of 3 ml. of haemolysate, 5 ml. of 0·185 mM-serotonin and 3 ml. of 10% (w/v) trichloroacetic acid, mixed in that sequence. Curve *B* is the spectrum obtained when the same quantities were mixed in the sequence haemolysate, trichloroacetic acid, serotonin. Extinction was measured in a Beckman DU spectrophotometer in 1 cm. cells, with a blank in which 5 ml. of water replaced the 5 ml. of serotonin. In this reaction there was a 74% loss of the added serotonin (spectrophotofluorimetric assay). Curve *A* has not been corrected for the remaining serotonin. If this were done that part of curve *A* in the region from 250–300 mμ would have a small decrease in extinction, and there would be no discernible change at wavelengths greater than 300 mμ.

(Numata, 1940), serotonin is slowly destroyed in the presence of denatured oxyhaemoglobin (Fig. 1, curve *A*).

Specificity of the reaction

Several analogues of serotonin were incubated with haemolysates of rat erythrocytes. Bufotenin (5-hydroxy-*NN*-dimethyltryptamine), 5-hydroxytryptophan and 5-HIAA all reacted as measured by fluorescence loss at λ_a 300, λ_f 555 mμ in 3 N-HCl. The ultraviolet spectra were similar to that shown in Fig. 4 for the oxidation product of serotonin. Bufotenin gave a product with the same pink colour as did serotonin, but 5-hydroxytryptophan and 5-HIAA yielded an orange–pink product. Tryptamine and indole-3-acetic acid were not affected by denaturing oxyhaemoglobin.

Nature of the reaction product(s)

Owing to the instability of the product, several attempts to isolate it were unsuccessful [e.g. Amberlite XE-64 resin in the ammonium form, Celite, paper chromatography (Rodnight, 1956) and paper electrophoresis]. The product is characterized by a loss of fluorescence both in 3 N-HCl at λ_a 300, λ_f 555 mμ and at pH 1·0–7·0 at λ_a 300, λ_f 360. No new fluorescent peaks were observed. There is also a loss of 5-hydroxyindole material as measured by the colorimetric method of Udenfriend, Weissbach & Clark (1955). The absorption spectrum (Fig. 4) changes both in the ultraviolet and in the visible. The pink colour fades in about half an hour at pH 5·0 and lasts for half a day in trichloroacetic acid. It changes to yellow instantly upon the addition of excess of NaOH, and disappears in a few minutes in 3 N-HCl. The pink product of serotonin oxidation was not extracted from the aqueous phase with benzene, chloroform,

light petroleum or carbon tetrachloride, but was extracted into butanol, in which the colour was stable for several days. The product of bufotenin oxidation remained in the aqueous phase with all the above-mentioned solvents including butanol. When a concentrated solution of serotonin was oxidized by concentrated denaturing oxyhaemoglobin, the filtrate was a turbid brownish pink which formed a black precipitate overnight. The addition of ascorbic acid to the pink product causes a rapid loss of colour, but the fluorescence does not return.

The product was tested for possible biological activity on the heart of the clam *Venus mercenaria*, with essentially the bioassay procedure of Twarog & Page (1953). No activity was found for the oxidized products of serotonin, bufotenin, 5-hydroxytryptophan or 5-HIAA.

DISCUSSION

The present results show that during the denaturation of oxyhaemoglobin there is a strong peroxidase-like activity which can attack 5-hydroxyindole compounds, as well as glutathione and ascorbic acid, as was found by Numata (1940). Rodnight (1958) has also noted that under certain conditions oxyhaemoglobin can destroy serotonin and he suggested that the process was similar to the oxidation of ascorbic acid and glutathione by denaturing oxyhaemoglobin. The product of this oxidation may be similar to a compound first observed by Cromartie & Harley-Mason (1957), who found that when oxygen is bubbled through a solution of 5:6-dihydroxy-2:3-dimethylindole, a pink product develops and the ultraviolet-absorption spectrum changes. The absorption spectrum of their product is similar to the spectrum of the oxidized product formed by the action of oxyhaemoglobin undergoing denaturation on serotonin (Fig. 4). It is thus possible that in the reaction with oxyhaemoglobin the 5-hydroxyindoles are first hydroxylated and then further oxidized. The formation of a pink product from serotonin is reminiscent of the oxidation of adrenaline solutions by atmospheric oxygen, and there may be some similarity in the structure of the serotonin oxidation product and compounds like adrenochrome (Harley-Mason, 1950; Beaudet, Debot, Lambot & Toussaint, 1951).

These results, in addition to providing further information on the oxidizing properties of haemoglobin undergoing denaturation, indicate that a system containing oxyhaemoglobin should be saturated with carbon monoxide before attempting to assay for 5-hydroxyindoles.

SUMMARY

1. Oxyhaemoglobin, when it is undergoing denaturation, can oxidize serotonin and several related 5-hydroxyindoles. The reaction is completely inhibited by carbon monoxide and partially inhibited by ascorbic acid.

2. The oxidized product(s) is characterized by a pink colour, a change in the ultraviolet-absorption spectrum and a loss of fluorescence.

This investigation was supported by Grant M1378 from the United States Public Health Service. We are grateful to Dr R. W. Gerard for helpful advice and criticism.

REFERENCES

Beaudet, C., Debot, F., Lambot, H. & Toussaint, J. (1951). *Experientia*, **7**, 293.
Cromartie, R. I. T. & Harley-Mason, J. (1957). *Biochem. J.* **66**, 713.
Drabkin, D. L. (1946). *J. biol. Chem.* **164**, 703.
Harley-Mason, J. (1950). *J. chem. Soc.* p. 1276.
Ling, N.-S. & Blum, J. J. (1958). *Fed. Proc.* **17**, 98.
Numata, I. (1940). *Biochem. Z.* **304**, 404.
Rodnight, R. (1956). *Biochem. J.* **64**, 621.
Rodnight, R. (1958). *J. Physiol.* **141**, 10P.
Stacey, R. S. (1956). *J. Physiol.* **132**, 39P.
Twarog, B. M. & Page, I. H. (1953). *Amer. J. Physiol.* **175**, 157.
Udenfriend, S., Bogdanski, D. F. & Weissbach, H. (1955). *Science*, **122**, 972.
Udenfriend, S., Weissbach, H. & Clark, C. T. (1955). *J. biol. Chem.* **215**, 337.
Willstätter, R. & Pollinger, A. (1923). *Hoppe-Seyl. Z.* **130**, 281.

The Isolation and Structure of Actinomycins II and III

By A. W. JOHNSON and A. B. MAUGER
Department of Chemistry, University of Nottingham

(*Received* 3 *April* 1959)

Since the discovery of actinomycin A by Waksman & Woodruff (1940) it has been recognized (Vining & Waksman, 1954) that the actinomycin complexes A, B and X consist of the same components, actinomycins I, II, III, IV and V, in different proportions and that actinomycin D is essentially pure actinomycin IV. The structure of actinomycin IV (I; A = B = L-proline) has been determined by Bullock & Johnson (1957). Recently, Katz, Pienta & Sivak (1958) have shown that the composition of