The anti-oxidant effect of serotonin*

When slices or homogenates of liver, kidney or brain are incubated aerobically, lipide peroxides are formed, which can be estimated by the thiobarbituric acid (TBA) method[1]. Peroxides are formed under these conditions as the result of the catalytic action of cytochrome hemoglobin, iron or ascorbic acid on the unsaturated lipides of the cell. On the other hand, homogenates of bone marrow or intestinal mucosa tissues in which active mitoses are occurring, do not produce lipide peroxides unless they are either exposed to ultraviolet light *in vitro* or taken from animals previously exposed to X-rays[2]. Peroxide formation is also inhibited in regenerating rat liver during the time of maximal mitotic activity[3] and in hepatomas[4]. Since lipide peroxides inhibit cell division in bacteria[5] and in marine worm eggs[6], these facts suggest that dividing cells are protected against peroxide formation by the presence of anti-oxidants, and these can be inactivated by irradiation. The presence of anti-oxidants in bone marrow and intestinal mucosa is shown by the fact that the addition of homogenates of these tissues to liver inhibits peroxide formation in the latter ([2] and unpublished experiments).

KOHN AND LIVERSEDGE[7] have shown that epinephrine acts as an anti-oxidant for peroxide formation in tissues. It is also known that certain sulfhydryl compounds particularly glutathione, are anti-oxidants under certain conditions, and that the concentration of sulfhydryl group is reduced in bone marrow after exposure of the animal to X-rays[8]. During a search for other naturally occurring anti-oxidants, we have found that serotonin (5-hydroxytryptamine) is a very powerful inhibitor of lipide peroxide formation in tissues.

Experimental. Two test systems were used. Rat brain was homogenized in 0.05 *M* Na-K phosphate buffer, pH 6.8 and diluted 1 to 20. One-half ml of this was added to 1.5 ml of buffer with or without various compounds to be tested and incubated for 40 minutes aerobically at 37°. Rat liver mitochondria were prepared in sucrose and washed with isotonic KCl, and the equivalent of 100 mg of wet tissue was incubated in 3.0 ml of isotonic KCl buffered with phosphate pH 7.4. Incubated 60 minutes alone, this preparation produced very little peroxide, but in the presence of 1.7 μmoles ascorbic acid, peroxides were rapidly formed. In both cases, the reaction was stopped by the addition of 1.0 ml 20% trichloroacetic acid, after which 1.0 ml of 0.75% TBA was added. The mixture was heated in a boiling water bath for 15 minutes, then centrifuged and the color was read in a colorimeter.

Fig. 1 shows the effect of a number of compounds on lipide peroxide formation in rat brain homogenates. Serotonin was a more active inhibitor than bufotenine (the N-dimethyl derivative of serotonin) and tryptamine. Epinephrine was much less active than these indole amines, and tyramine, dopa, mescaline and indole acetic acid were essentially without effect in the concentration range used.

Serotonin inhibited peroxide formation in the presence of marsilid, which inhibits the amine oxidase, and also in boiled brain homogenates. The intact molecule is therefore active; however,

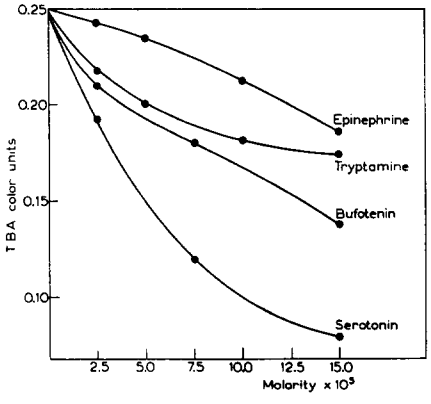

Fig. 1. The effect of various compounds on lipide peroxide formation in rat brain homogenates diluted 1 to 20 with no added ascorbic acid. Incubated 40 minutes at 37°; pH 6.8. The horizontal axis represents the TBA value of the homogenate at zero time.

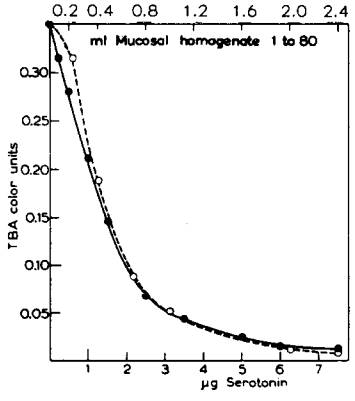

Fig. 2. The effect of different amounts of serotonin (solid line) and of rat intestinal mucosa diluted 1 to 80 (dotted line) on lipide peroxide formation in rat liver mitochondria catalyzed by 1.7 μmoles of ascorbic acid. Incubated 60 minutes at 37°; pH 7.4; reaction vol., 3.0 ml.

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after incubation with the amine oxidase the resultant aldehyde had approximately the same activity as serotonin. Peroxide formation in 3.0 mg of pure methyl linolenate emulsified with a detergent and incubated with cytochrome *c*, was completely inhibited by 10 μg/ml of serotonin.

Fig. 2 shows the effect of several concentrations of serotonin on peroxide formation in liver mitochondria, catalyzed by ascorbic acid, and the similar effect of a homogenate of rat intestinal mucosa diluted 1 to 80. The anti-oxidant activity of the mucosa cannot be entirely accounted for by its serotonin content since the concentration in the intestine would have to be 250 μg/g, which is much higher than the values given by ERSPAMER[9]. Preliminary studies have shown that the main anti-oxidant in the mucosa is non-dialyzable and acid-labile; apparently a large molecule may be acting as a chelating agent or as a carrier of an active compound.

It is not possible to state that the anti-oxidant activity of serotonin explains any of its physiological actions. It might be of some importance in the radiation protection given by serotonin when it is injected immediately before exposure[10]. Other hydroxy-indoles may prove to be more active protective agents.

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1 F. BERNHEIM, M. L. C. BERNHEIM AND K. M. WILBUR, *J. Biol. Chem.*, 174 (1948) 257.
2 F. BERNHEIM, A. OTTOLENGHI AND K. M. WILBUR, *Radiation Research*, 4 (1956) 132.
3 N. WOLFSON, K. M. WILBUR AND F. BERNHEIM, *Exptl. Cell. Research*, 10 (1956) 556.
4 S. K. DONNAN, *J. Biol. Chem.*, 182 (1950) 415.
5 E. STILLWELL, (unpublished).
6 K. M. WILBUR, C. B. KENASTON, N. WOLFSON, A. OTTOLENGHI AND M. E. GAULDEN, *Anat. Record*, 120 (1954) 708.
7 H. I. KOHN AND M. LIVERSEDGE, *J. Pharmacol. Exptl. Therap.*, 82 (1944) 292.
8 C. LUTWAK-MANN, *Biochem. J.*, 52 (1952) 356.
9 V. ERSPAMER, *Pharmacol. Revs.*, 6 (1954) 425.
10 J. L. GRAY, J. T. TEW AND H. JENSEN, *Proc. Soc. Exptl. Biol. Med.*, 80 (1952) 604.

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A convenient method for the esterification of amino acids and their derivatives

During the study of the biochemistry of the proteolytic enzymes the need often arises for the methyl esters of amino acids as artificial substrates. However, in a laboratory where preparative organic chemistry is not regularly practised, the Fischer-Speier method may be difficult and time-consuming, especially in the maintenance of the apparatus for producing dry hydrogen chloride and in using it as a catalyst in the reaction. We have found that the reaction may be carried out more easily when a strong cation-exchange resin, *e.g.* Amberlite IR-120(H) or Zeo-Karb 225(H) is substituted as catalyst.

The resin was prepared by treating it with 2 to 4 bed-volumes of 2 *N* HCl, washing with distilled water until free from acid and drying it over $CaCl_2$ *in vacuo*. The esterification of hippuric acid, for example, is carried out as follows. 10 g hippuric acid, 10 g of the resin and 300 ml of dry methanol are refluxed for three hours with constant stirring. The resin is then removed by filtration and washed with methanol. The combined filtrate and washings are then concentrated to a syrup *in vacuo* and the residue shaken with aqueous sodium carbonate. In some instances the ester crystallises out at this point, but whether this happens or not the mixture is extracted three times with 50 ml of ether. The extract is dried over anhydrous sodium sulphate and the ether removed *in vacuo*. On cooling, the ester crystallises and our preparations had a melting point of 75–77° before and after two recrystallisations from 50:50 ethanol:water. HERZIG AND LANDSTEINER[1] report 75° for the melting point of the methyl ester of hippuric acid. The average yield of ester is 70%. It should be noted that once the resin has been prepared for use it may be stored almost indefinitely in a tightly stoppered container.

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1 J. HERZIG AND K. LANDSTEINER, *Biochem. Z.*, 61 (1914) 463.

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