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Serotonin-Induced Swelling of Rat Liver Mitochondria *

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Synopsis

Serotonin is a potent inducer of swelling of isolated rat liver mitochondria. The dependence of the magnitude of serotonin creatinine sulfate-induced swelling upon the inducer concentration was evident down to 5 x $10^{-6}M$. Possibility of heavy metal contamination was excluded by the use of ash obtained after combustion of the serotonin preparation employed. Serotonin hydrochloride prepared by a novel Sephadex G-10 column method was shown to induce mitochondrial swelling. Sulfate or creatinine sulfate enhanced the swelling activity of serotonin. The time-course of serotonin-induced swelling simulated that of the swelling induced by NAD-linked substrate of mitochondrial oxidation. On a molar basis, serotonin was found to be more active than the NAD-linked substrates or some of the related indole derivatives. NAD-linked substrates interacted synergistically, and Ca^{2+} interacted additively with serotonin in the induction of swelling. Succinate markedly counteracted the serotonin effect. Serotonin-induced swelling was reversed by ATP, without an requirement of extraneous Mg^{2+}. It was prevented by serum albumin, rotenone, amytal, antimycin A, dinitrophenol, and cyanide, but could not be prevented by a monoamine oxidase inhibitor β-isopropylphenylhydrazine. It differed from ascorbate-induced lysis in both the time course and magnitude. Ascorbate-induced mitochondrial lysis was prevented by serotonin. Dependence of serotonin-induced swelling on the NAD-branch of the respiratory chain was proposed and possible mechanism of the action was discussed.

Serotonin can be regarded as something like a hormone and the enterochromaffin cells as the "diffuse endocrine organ" producing it. The classical methods in endocrinology to reveal the role of a hormone are, however, poorly applicable in the case of the enterochromaffin cells. It has been shown that on some humoral controling factors *in vitro* observations are of use in studying their possible mode of action, even if the finding may have inherent limitations in drawing physiological deductions. The studies on thyroxine (Lindberg *et al.*, 1961; Scott and Hunter, 1966) and parathormone (Utzumi *et al.*, 1966) suggested that effects of some hormones reside in their capacity to alter mitochondrial morphology and function (Tepperman and Tepperman, 1960 ; Lehninger, 1962).

During the course of studies on the effect of serotonin on carbohydrate metabolism in rats (Ui and Kobayashi, 1962), it was found that serotonin creatinine sulfate *in vitro* affected the oxidation of NAD-linked substrates by liver mitochondria, and depressed the P : O ratio with α-ketoglutarate as substrate (Warashina, 1967). These effects were shown to be time-dependent, and bear a certain similarity in nature to the effect of thyroxine. Thus it

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seemed interesting to study whether serotonin acts primarily on the morphological integrity of isolated mitochondria, as detailed by previous workers on thyroxine or parathormone.

The data in the present report indicate that serotonin creatinine sulfate is a potent inducer of swelling in isolated rat liver mitochondria, and that the swelling is dependent upon mitochondrial electron transport.

Materials and Methods

Mitochondria were isolated essentially as described by Lehninger (1959), except for omission of the third washing from the livers of well-fed male Wistar-Imamichi rats. A suspension in 0.25*M* sucrose, 1.0 cc of which contained the mitochondria derived from 200 to 300 mg of wet liver, was made and used immediately.

Swelling and contraction were followed by measuring absorbancy changes of mitochondrial suspensions at 520 mμ in 10-mm^2 glass cuvettes, containing 0.1 cc of the mitochondrial suspension and 3.2 cc of 0.125*M* KCl-0.02*M* Tris-HCl buffer, pH 7.4, with various other additions as shown in the figure legends. Cuvettes were incubated at 20°C in a water bath, and the readings were taken at given times as quickly as possible. Post-additions of reagents were made in 0.05 cc in order to minimize absorption changes caused by dilution. All solutions were brought to pH 7.4 just before use.

Glass-redistilled water was used for the preparation of all solutions, and the reagents were of highest commercial quality. Unless otherwise specified, serotonin was used as its creatinine sulfate salt. A novel Sephadex G-10 column method was used for preparation of serotonin hydrochloride. Sulfate and creatinine were easily separated from the complex upon elution with water. Serotonin, owing to its greater affinity for the gel bed, was retained, and was obtained only after the elution with 0.02*N* HCl. The resultant serotonin hydrochloride solution was lyophilized in the dark. The UV spectrum of this preparation agreed well with that of the original serotonin creatinine sulfate. The concentration of the final serotonin hydrochloride solution was adjusted according to the result of either fluorometric or UV absorption determinations.

Results

Specificity of serotonin-induced swelling

Serotonin, when added to mitochondrial suspensions, induced a definite swelling of large amplitude as reflected in the decrease of optical density at 520 mμ (Fig. 1). The dependence of the response upon dosage was noticeable down to $5 \times 10^{-6}M$, and no effect was detected when $5 \times 10^{-7}M$ was reached. The nature and the magnitude of swelling were roughly comparable to those induced by the NAD-linked substrates of tricarboxylic acid cycle at the dosage level of $10^{-3}M$. It can be seen that swelling induced by creatinine sulfate complex of serotonin (up to $5 \times 10^{-4}M$) was accompanied by a lag period averaging 10 mins., which became less evident when the concentration was raised to $1 \times 10^{-3}M$.

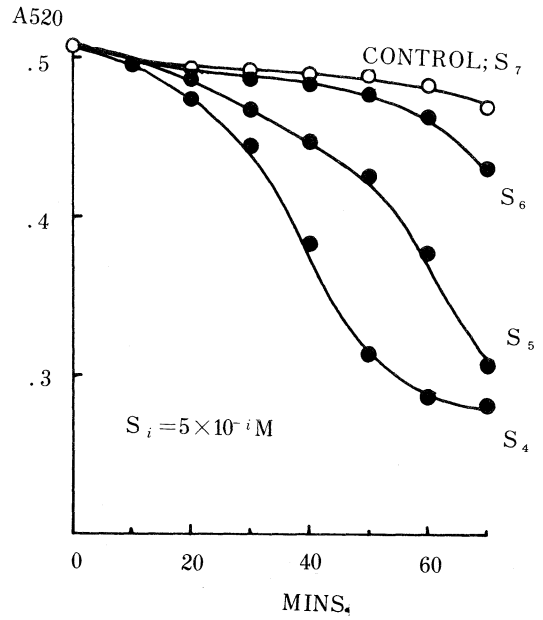

Fig. 1. Dosage-response relationship of serotonin-induced swelling. The test system contained 3.2 cc of 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4, and serotonin creatinine sulfate concentrations shown. Mitochondria added last at time zero in 0.1 cc.

The possibility that the swelling was induced by some contaminants of the serotonin creatinine sulfate complex hereby used, or even by sulfate and/or creatinine, was tested. A weighed sample of serotonin creatinine sulfate was taken into a platinum boat, and heated to

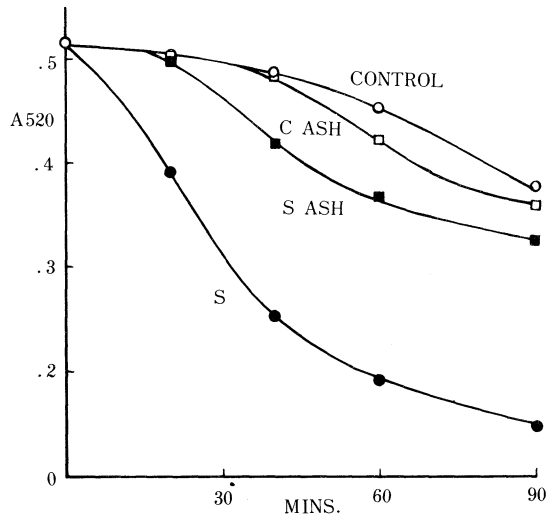

Fig. 2. Test of heavy metal contamination. Medium was 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4. S: serotonin creatinine sulfate $5 \times 10^{-4}M$. S ASH: ash obtained after combustion of serotonin creatinine sulfate. Equivalent amount to give the original concentration of $5 \times 10^{-4}M$ was added. C ASH: ash obtained after blank test for the ashing procedure. Equivalent amount was added. This figure represents the highest swelling capacity of serotonin-derived ash among 4 similar experiments.

combustion. Ash, if any, was dissolved into a small volume of water, and aliquots were used as additions in the swelling experiment. No appreciable swelling was observed by the addition of the ash solution, equivalent to as much

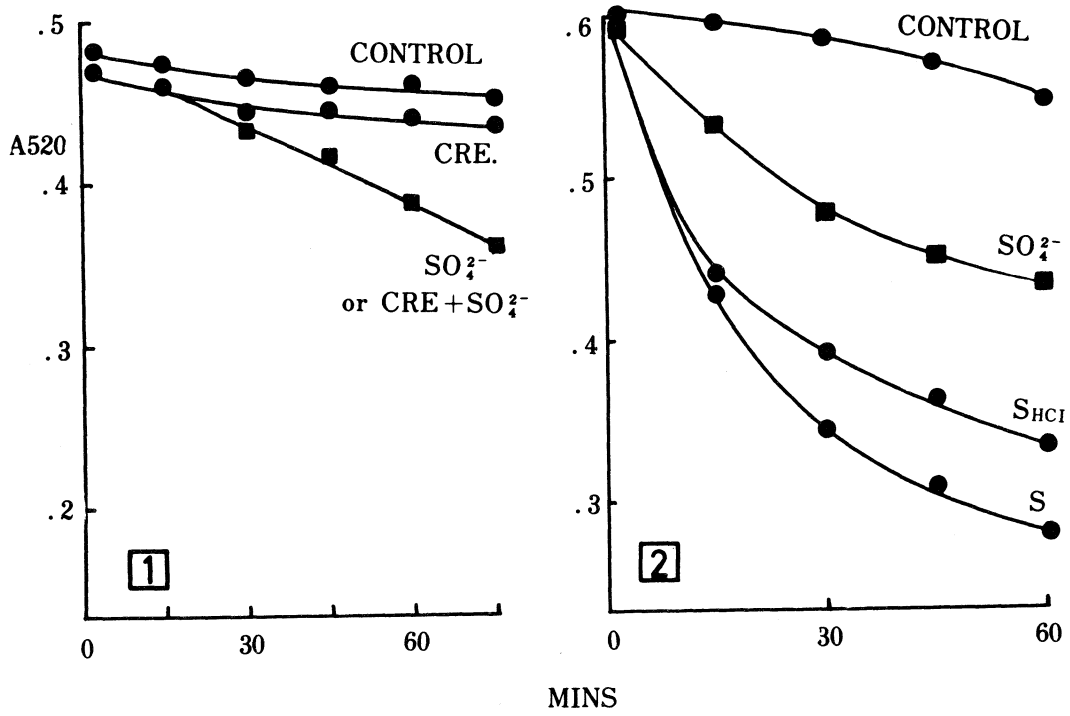

Fig. 3. Effect of creatinine and sulfate. Test medium was 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4. 1. CRE: creatinine $5 \times 10^{-4}M$. SO_4^{2-}: sodium sulfate $5 \times 10^{-4}M$. 2. S_{HCl}: serotonin hydrochloride prepared by Sephadex column method, $1 \times 10^{-3}M$. SO_4^{2-}: potassium sulfate $1 \times 10^{-3}M$. S: serotonin creatinine sulfate, $1 \times 10^{-3}M$.

as 5 x $10^{-4}M$ serotonin creatinine sulfate (Fig. 2). A possible contamination of heavy metal ions as swelling inducer was thus unlikely. Creatinine itself induced no appreciable swelling at a concentration of 5 x $10^{-4}M$ (Fig. 3.1). On the other hand, potassium sulfate as well as creatinine sulfate at 5 x $10^{-4}M$ or higher concentrations induced some swelling. The swelling induced by sulfate was studied separately, and the result will be described elsewhere (Watanabe and Kobayashi, 1969). However, the extent of the sulfate-induced as well as creatinine sulfate-induced swelling was by no means as large as that induced by serotonin creatinine sulfate complex.

Serotonin hydrochloride, prepared by the Sephadex method, as well as serotonin oxalate (not shown in figure) induced mitochondrial swelling (Fig. 3.2). It seems evident that primarily serotonin molecule itself is required for the induction of mitochondrial swelling, even though the extent of swelling induced by serotonin hydrochloride was a little smaller than that produced by the equivalent amount of serotonin creatinine sulfate complex. Preparation method of serotonin hydrochloride, as reported by Bogdanski *et al.* (1956) was found to be unsuitable for the swelling experiment. The blank preparation of Bogdanski extraction procedure was found to induce a notable swelling. Furthermore, by unknown reasons, the swelling experiments using Bogdanski-type of serotonin hydrochloride preparations were met with a variability of the results.

It appeared possible that metabolic transformations of the serotonin molecule are directly involved in induction of the swelling. Figure 4 shows that the degradation of serotonin by the monoamine oxidase system is not necessary to induce swelling. It is seen that the preincubation of mitochondria with β-isopropylphenylhydrazine (5 x $10^{-4}M$) for 30 mins., a condition which had been shown to result in a nearly complete inhibition of mitochondrial monoamine oxidase activity, failed to prevent the serotonin-induced swelling. It was observed

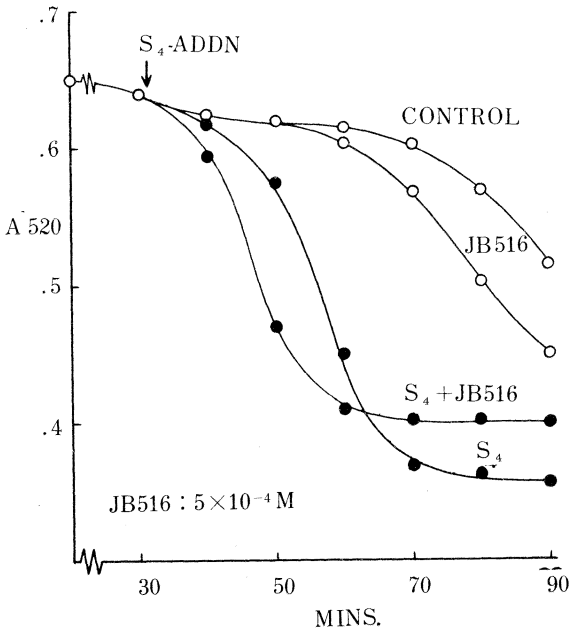

Fig. 4. Failure of monoamine oxidase inhibitor to prevent serotonin-induced swelling. Mitochondrial suspension was preincubated for the first 30 mins. with and without 5 x $10^{-4}M$ β-isopropylphenylhydrazine (JB516). At time shown by arrow, serotonin or its equivalent was added quickly. Final concentration of serotonin was 5 x $10^{-4}M$ (S_4).

that β-isopropylphenylhydrazine itself exhibited a slight swelling, and that in the presence of both β-isopropylphenylhydrazine and serotonin the onset of swelling was even faster than when serotonin alone was added to the medium. Acceleration by β-isopropylphenylhydrazine of the early stage of serotonin-induced swelling was confirmed by the experiments using lower concentrations of this agent, *e.g.*, 1 x$10^{-4}M$, at which this agent itself exerted minimal effect on mitochondrial morphology. On the other hand, the later stage of serotonin-induced swelling was slightly but significantly affected by β-isopropylphenylhydrazine. The plateau level of the serotonin plud β-isopropylphenylhydrazine-induced swelling remaiend higher than that of the serotonin-induced one. It

is possible that the later stage of serotonin-induced swelling is dependent upon the conversion into 5-hydroxyindoleacetaldehyde. However, the exact mechanism is still uncertain since β-isopropylphenylhydrazine was also shown to inhibit spontaneous swelling in the later stage.

Specific participation of serotonin in the induction of swelling was confirmed by an experiment in which the effect of equimolar concentration of serotonin and substrates of mitochondrial oxidation were compared. The result shown in Figure 5 clearly demonstrates that at a concentration level of $5 \times 10^{-4}M$, serotonin was the only agent tested causing substantial swelling of mitochondria, and that neither the NAD-linked substrate of respiration nor succinate was capable of producing noticeable effect. The swelling action of serotonin can not solely be accounted for by the feeding of electrons from NADH, which can be produced by the aldehyde dehydrogenase reaction, into the flavoprotein-cytochrome system of mitochondria.

Serotonin is relatively unique among a series of indole derivatives tested for swelling activity. At a concentration level of $5 \times 10^{-4}M$ neither 5-hydroxytroytophan nor 5-hydroxyindoleacetic acid (as cyclo hexylammonium salt) exerted substantial swelling promotion. L-Tryptophan and indoleacetic acid, on the

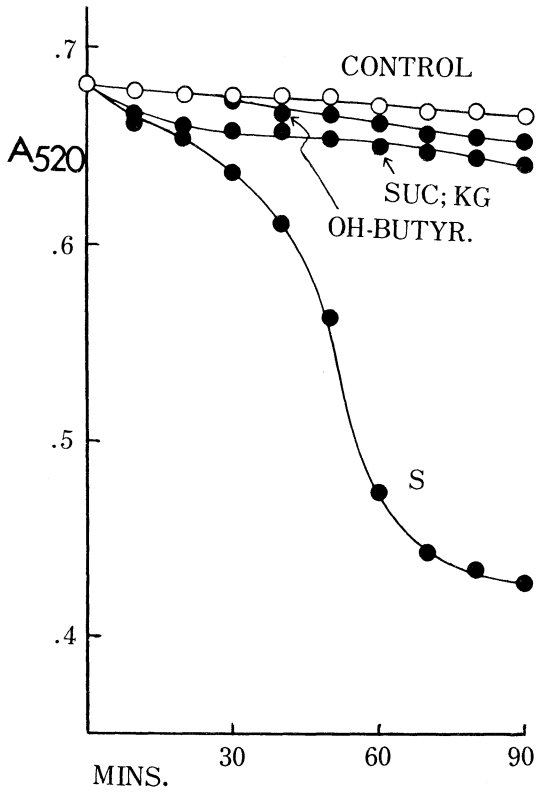

Fig. 5. Specificity of serotonin-induced swelling. 1. Comparison with substrates of mitochondrial oxidation. Addition was $5 \times 10^{-4}M$ serotonin (S), succinate (SUC), φ-ketoglutarate (KG), or β-hydroxybutyrate (OH-BUTYR.)

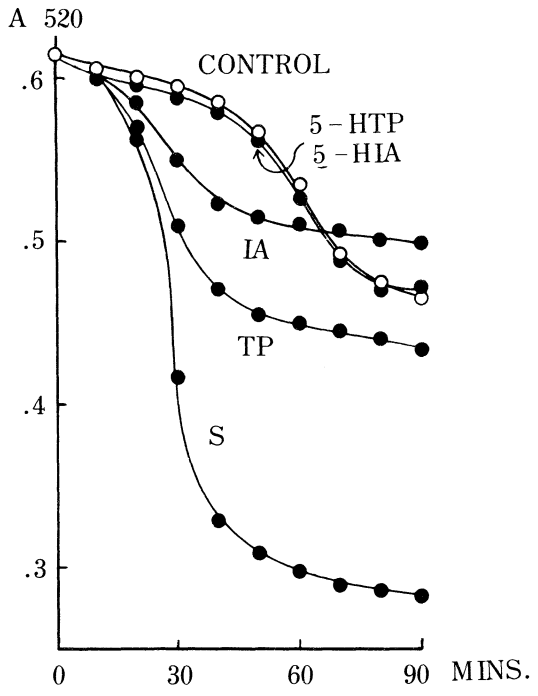

Fig. 6. Specificity of serotonin-induced swelling. 2. Comparison with related indole derivatives. Addition was $5 \times 10^{-4}M$ serotonin (S), 5-hydroxytryptophan (5-HTP), 5-hydroxyindoleacetic acid as cyclohexylammonium salt (5-HIA), L-tryptophan (TP), or indoleacetic acid (IA). Mitochondria added last at time zero.

other hand, were found to cause swelling, but their effects were only slight when compared with that of serotonin (Fig. 6). It is highly probable that the molecular structure of 5-hydroxytryptamine, *per se*, is needed for the promotion of mitochondrial swelling.

Figure 7 illustrates the difference between the serotonin-induced swelling and ascorbate-induced lysis of mitochondria. The lag period of ascorbate-induced lysis was longer, and the plateau level reached was much lower than the serotonin-induced swelling. These findings were confirmed even at higher concentrations of ascorbate where the lytic effect was less. Furthermore, when both serotonin and ascorbate were present, the ascorbate-induced lysis was prevented while serotonin-induced swelling occurred almost normally. These results support the view that the serotonin-induced swelling is distinct from the ascorbate-induced lytic phenomenon as described by Hunter (1961). Colorimetric analysis of lipid peroxide by thiobarbiturate method revealed that inhibition of mitochondrial lysis was accompanied by an inhibition of lipid peroxide liberation from mitochondria.

Interaction of serotonin with other inducers

The initial stage of the swelling induced by α-ketoglutarate was accelerated by the presence of serotonin. Figure 8 shows the result of a typical experiment in which 5 x $10^{-4}M$ serotonin enhanced the rate of swelling caused

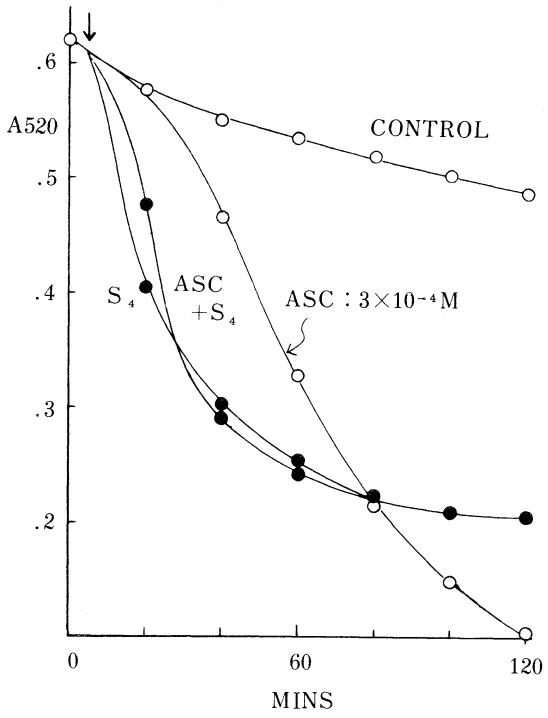

Fig. 7. Comparison and interaction of serotonin-induced swelling with ascorbate-induced lysis. Test medium was 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4. Addition of 5 x $10^{-4}M$ serotonin (S_4) and/or 3 x $10^{-4}M$ ascorbate (ASC) was made at time shown by arrow.

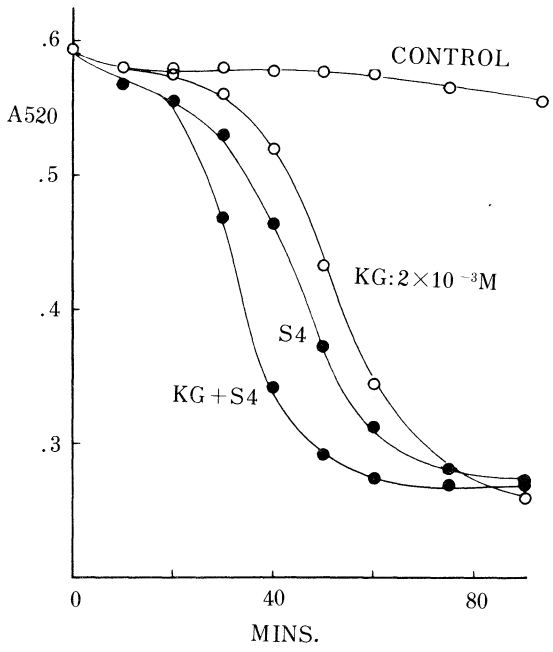

Fig. 8. Synergistic interaction of serotonin with α-ketoglutarate. Swelling was induced by 5 x $10^{-4}M$ serotonin (S_4) and/or 2 x $10^{-3}M$ α-ketoglutarate (KG). Mitochondria added last at time zero.

by 2 x $10^{-3}M$ α-ketoglutarata. The plateau of the optical density reached was, however, practically identical with that produced by α-ketoglutarate alone. It will be seen that the effect of serotonin was also accelerated by the presence of α-ketoglutarate, and that their interaction was synergistic rather than purely additive.

Tests of serotonin together with inorganic phosphate (2 x $10^{-3}M$) showed the closest analogy with that just described, *i.e.*, each accelerated the response of the other without alteration of the final magnitude. The synergistic acceleration by inorganic phosphate of the rate of serotonin-induced swelling was observed at graded serotonin concentrations of 5 x 10^{-4}, 5 x 10^{-5}, and 5 x $10^{-6}M$.

The interaction of serotonin and succinate, whose oxidation is not linked to NAD, deserves attention. A typical result shown in Figure 9.1 shows that succinate at the concentration level of 1 x $10^{-3}M$, which in itself elicited negligible effects on the optical density of mitochondrial suspension, markedly inhibited the serotonin-induced swelling. Succinate did induce a definite swelling in the presence of amytal (1 x $10^{-3}M$), and in Figure 9.2 it is shown that the swelling was inhibited by malonate(1 x $10^{-2}M$). The result in Figure 9.3 demonstrates that malonate was

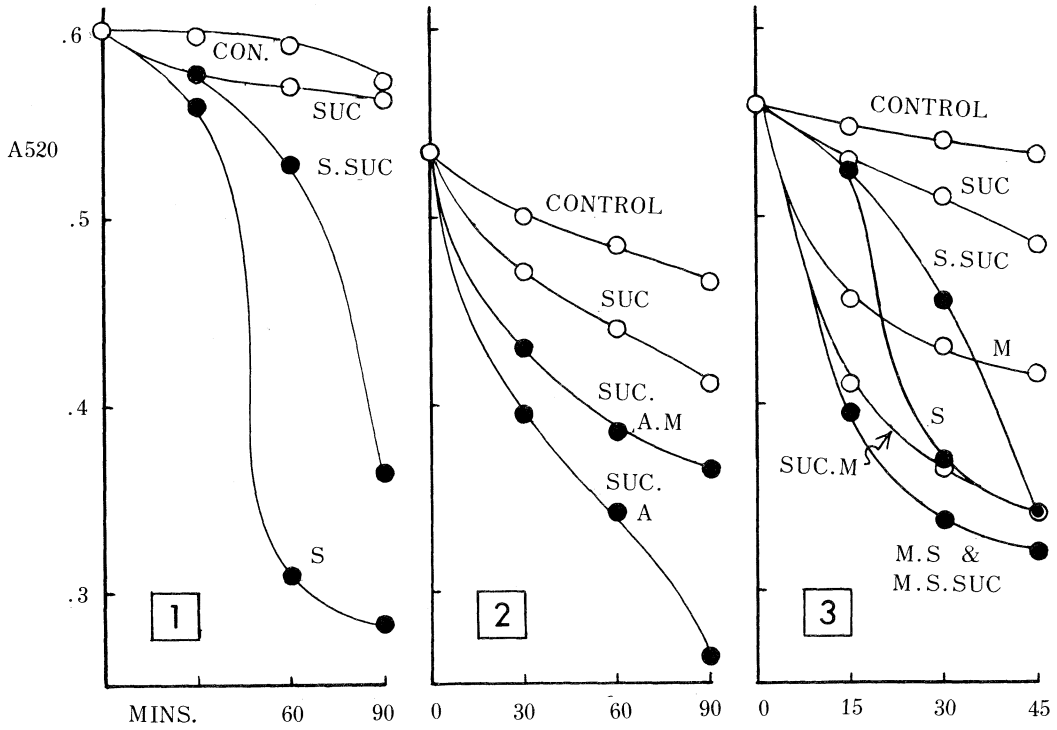

Fig. 9. Interactions of serotonin, succinate, amytal, and malonate in mitochondrial swelling. 1. Inhibition of serotonin-induced swelling by succinate. Test media contained 5 x $10^{-4}M$ serotonin (S) and/or 1 x $10^{-3}M$ succinate (SUC) as indicated. 2. Potentiation of succinate-induced swelling by amytal, and inhibitory effect of malonate on it. Test media contained 2 x $10^{-3}M$ succinate (SUC), 1 x $10^{-3}M$ amytal (A) and/or 1 x $10^{-2}M$ malonate (M) as indicated. 3. Malonate effect on serotonin and/or succinate-induced swelling. Test media contained 5 x $10^{-4}M$ serotonin (S), 2 x $10^{-3}M$ succinate (SUC), and/or 1 x $10^{-2}M$ malonate (M) as indicated.

also capable of preventing the inhibitory action of succinate ($2 \times 10^{-3}M$) in the serotonin-induced swelling. It seems quite probable that the observed inhibitory action of succinate is mediated through the oxidation by succinic dehydrogenase. However, the effect of malonate on swelling phenomena can not solely be accounted for by the competitive inhibition of succinate oxidation. Malonate induced a notable swelling, and stimulated the serotonin-induced as well as the succinate-induced swelling.

It was further shown that swelling induced by β-hydroxybutyrate at a concentration of $5 \times 10^{-3}M$ was inhibited by the presence of $2 \times 10^{-3}M$ succinate (Fig. 10.1). When the concentration of the inducer was lowered to $5 \times 10^{-4}M$, the effect of β-hydroxybutyrate itself was minimal, and the presence of succinate exerted either no effect or a slightly stimulative effect (Fig. 10.2).

The above finding seems to lend support to the postulation that the electron transfer via NAD-cytochrome system is involved in the serotonin-induced swelling mechanism.

Since the *in vivo* effects of serotonin have

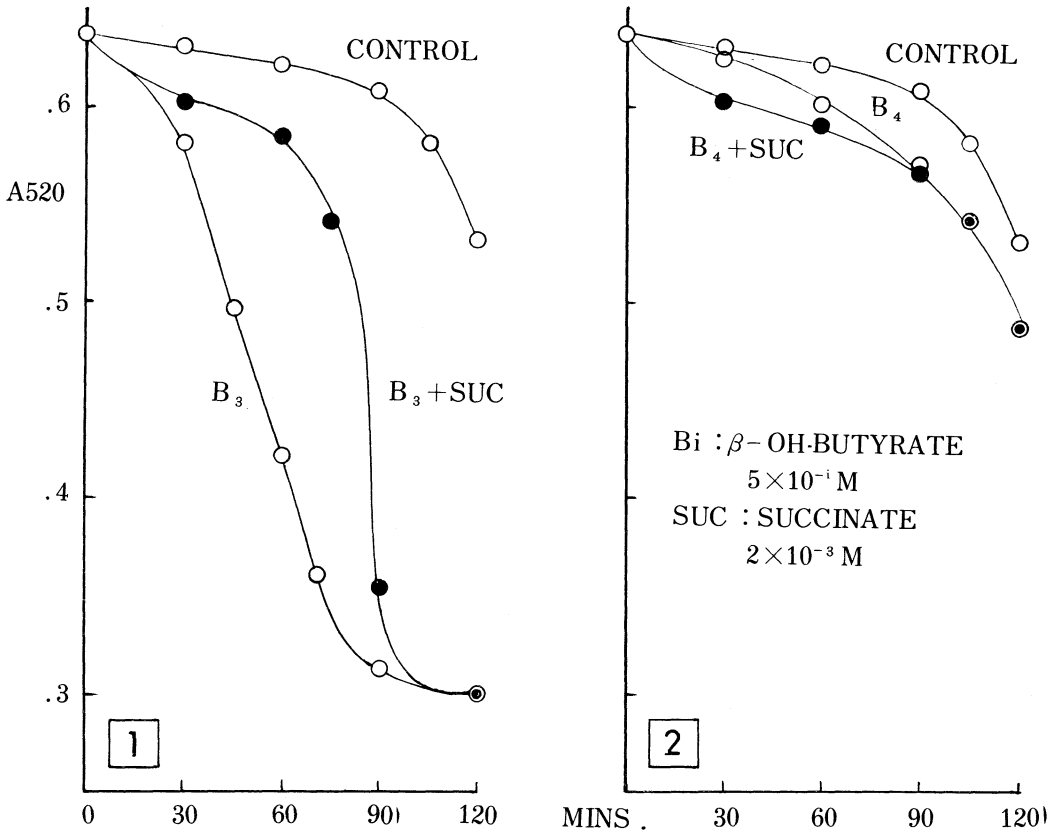

Fig. 10. Interaction of succinate with β-hydroxybutyrate. 1. Inhibition of β-hydroxybutyrate-induced swelling by succinate. Swelling was induced by $5 \times 10^{-3}M$ β-hydroxybutyrate (B_3). 2. Potentiation of β-hydroxybutyrate-induced swelling by succinate. Swelling was induced by $5 \times 10^{-4}M$ β-hydroxybutyrate (B_4). Succinate (SUC), when present, at $2 \times 10^{-3}M$. Mitochondria added last at time zero.

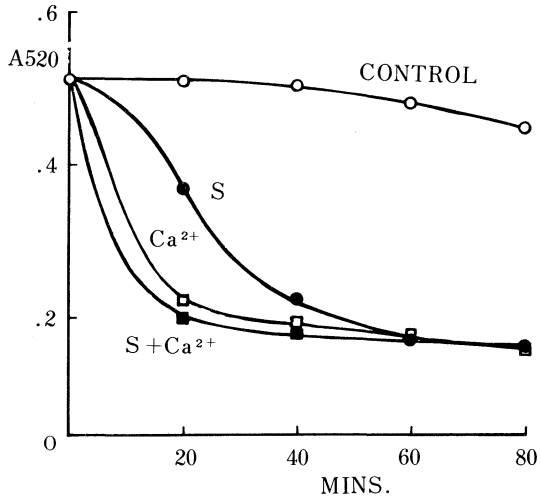

Fig. 11. Interaction of Ca^{2+} and serotonin. Test medium contained calcium chloride (Ca^{2+}) 1 x $10^{-3}M$ and/or serotonin (S) 5 x $10^{-4}M$ as indicated.

been correlated to dislocation of Ca^{2+} ions in tissues (Woolley, 1962), it seemed of interest to study the interaction of both agents on mitochondrial swelling. It will be seen in Figure 11 that these two agents interacted additively during the earlier phase of swelling induction and that the same plateau level of serotonin-induced swelling was reached afterwards either in the presence and absence of Ca^{2+} ions. Various concentration pairs were tried, and the above relationship was held throughout.

Reversal and inhibition of serotonin-induced swelling

Adenosine triphosphate caused an contraction of mitochondria swollen by serotonin (5 x $10^{-4}M$). Determinations of inorganic

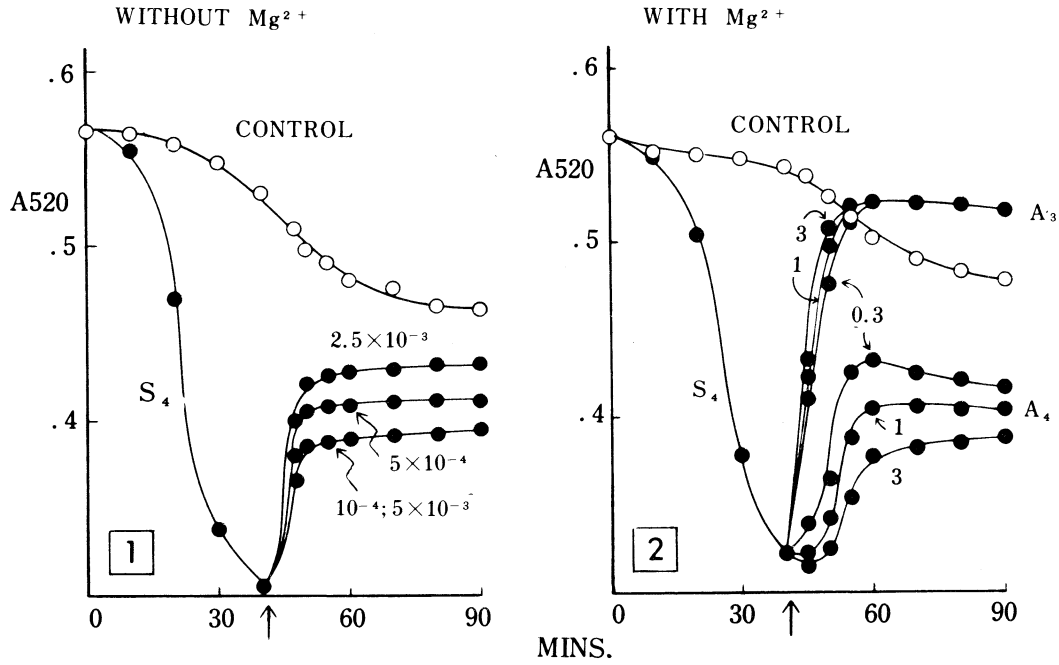

Fig. 12. Reversal of serotonin-induced swelling by ATP. 1. Without extra Mg^{2+}. Swelling was induced by 5 x $10^{-4}M$ serotonin at zero time. At time shown by arrow ATP was added to make the final molar concentration cited in the figure. 2. With extra Mg^{2+}. Swelling was induced by 5 x $10^{-4}M$ serotonin. At time shown by arrow additions were made to yield the following final concentrations: ATP, 5 x $10^{-4}M$ (A_4) or 5 x $10^{-3}M$ (A_3); Mg^{2+}, 3 x $10^{-4}M$ (0.3), 1 x $10^{-3}M$ (1), or 3 x $10^{-3}M$ (3). Test medium was 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4.

phosphate in the medium revealed that the contraction was accompanied by an increase of phosphate liberation, presumably brought about by the hydrolytic cleavage of ATP. The magnitude of contraction was roughly parallel to the final concentration of ATP until 2.5 x $10^{-3}M$ was reached. The higher ATP concentration of 5 x $10^{-3}M$, however, produced a smaller reversal, and the recovered plateau level was approximately equal to that brought about by 1 x $10^{-4}M$ of ATP (Fig. 12.1).

The importance of the relative concentration of ATP and Mg^{2+} in the contraction of serotonin-treated mitochondria is shown in Figure 12.2. When the final ATP concentration was 5 x $10^{-3}M$, the presence of Mg^{2+} greatly augmented the contraction. The rate of recovery was parallel to the final concentration of Mg^{2+} as far as the present experimental conditions of 3 x $10^{-4}M$, 1 x$10^{-3}M$ and 3 x $10^{-3}M$ were concerned. The recovered plateau levels were virtually the same in these cases, and approximately 80 per cent recovery of the initial level was obtained. Thus the reversal was more than twice that of the no-Mg^{2+} experiment. On the other hand, when the final ATP concentration was 5 x $10^{-4}M$, a reversal of the relationship between Mg^{2+} concentration and magnitude of contraction was observed. When compared with the no-Mg^{2+} experiments, the recovery was far less at 3 x $10^{-3}M$ of Mg^{2+}, and slightly less at 1 x $10^{-3}M$. At 3 x $10^{-4}M$, however, Mg^{2+} favored the reversal slightly and temporarily. The quantitative consideration on the need of Mg^{2+} for ATP-induced contraction of serotonin-treated mitochondria requires further experiment, using exclusively deionized, quarz-distilled water and EDTA-treated mitochondrial preparation.

Figure 13 shows that serum albumin is a potent inhibitor of serotonin-induced swelling. The protective action of albumin against the swelling was demonstrable at a concentration as low as 0.6 mg per cc, without an addition of ATP or Mg^{2+}. Post-additions of albumin into serotonin-treated, partly swollen mitochondria were also effective in slowing down the rate of swelling, even though their effects were not as marked as those of pre-additions. It seems highly probable that an accumulation of fatty acids within mitochondria is involved in the serotonin-induced swelling. On the other hand, it was also found that albumin combined with a part of the serotonin present in the medium. Fluorometric determinations were carried out by the method of Udenfriend and others (1954), on the supernatants of $ZnSO_4$-$Ba(OH)_2$-deproteinized swelling medium from which mitochondria had been omitted. Under these conditions albumin was found to combine with 35 to 40 per cent of serotonin present in the medium when mitochondria were absent. Ultraviolet absorption data also supported the occurrence of such bindings. These findings show that at least a part of the protective action of albumin against serotonin-induced swelling may be ascribed to the for-

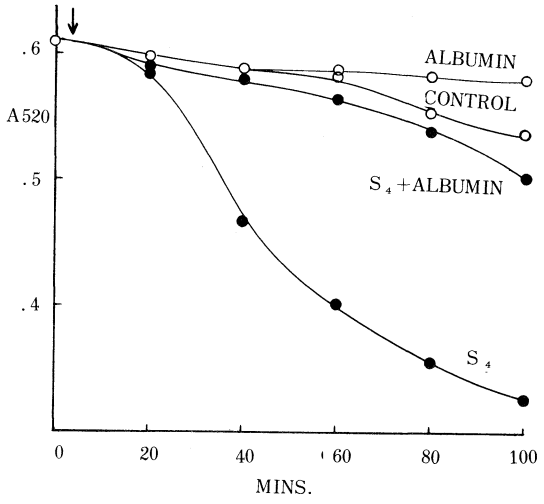

Fig. 13. Protective action of serum albumin against serotonin-induced swelling. Test medium was 0.125*M* KCl-0.02*M* Tris-HCl, pH 7.4, with and without bovine serum albumin (0.6 mg per cc). At time shown by arrow 5 x $10^{-4}M$ serotonin (S_4) or its equivalent was added quickly.

mation of a complex, leading to the decrease of free serotonin from the medium. Probably serotonin is only loosely bound to albumin, because no difference in the recoverable serotonin was observed between the albumin-containing and the control medium when the deproteinization steps were omitted.

A typical result in Figure 14 shows that rotenone and amytal markedly inhibited the serotonin-induced swelling. When the concentration of the inducer was kept constant at $5 \times 10^{-4}M$, rotenone block was nearly complete at $4 \times 10^{-7}M$, and amytal block was obtained at $2 \times 10^{-3}M$. Closer analysis of the data revealed that the action of these inhibitors was biphasic. A slight, transitory stimulation, which was followed by a sustained inhibition, was observed during the lag period of serotonin action.

Antimycin A, at $2.5 \times 10^{-7}M$, also inhibited the serotonin-induced swelling (Fig. 14.3). This inhibition was also preceded by a transitory acceleration of swelling quite similar to that observed in the amytal experiment. Higher concentrations of antimycin A (*e.g.*, $1 \times 10^{-6}M$) tended to induce swelling by themselves, although they inhibited the later phase of serotonin-induced swelling consistently. On the other hand, lower concentrations of antimycin A (*e.g.*, $1 \times 10^{-8}M$) definitely accelerated the initial phase of swelling induced by serotonin, and only slightly inhibited the later phase.

Figure 15 shows that dinitrophenol ($1 \times 10^{-4}M$) and cyanide ($1 \times 10^{-3}M$) are potent inhibitors of serotonin-induced swelling. A transitory swelling promotion by dinitrophenol was concentration-dependent, and a choice of optimum concentration clearly demonstrated the inhibitory effect.

The effects of these inhibitors can safely be ascribed to the block of electron flux

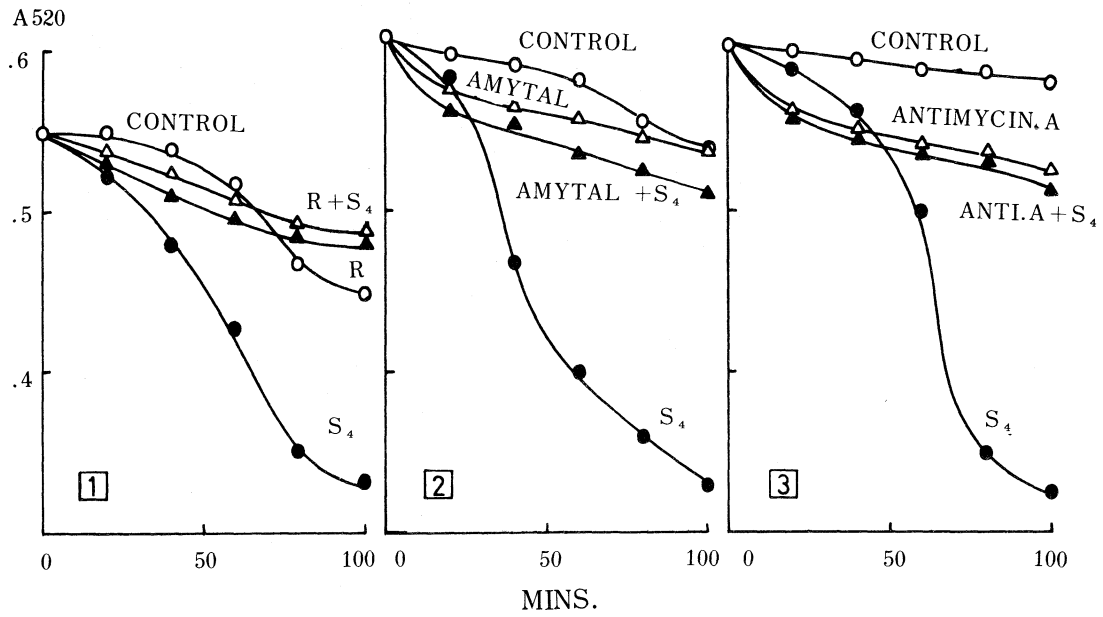

Fig. 14. Prevention of serotonin-induced swelling by respiratory chain inhibitors. Serotonin ($5 \times 10^{-4}M$, S_4), when present, added last at time zero. Other additions were, 1. rotenone (R) $4 \times 10^{-7}M$; 2. amytal, $1 \times 10^{-3}M$; 3. antimycin A, $2.5 \times 10^{-7}M$.

coupled with phosphorylation, even though the mechanism of the initial stimulative phenomena elicited by them needs further clarification.

Discussion

The experiments detailed above show that serotonin, a physiological intracellular component of a variety of animal species, induces a substantial swelling of rat liver mitochondria *in vitro*, at a concentration level as low as $10^{-6}M$. Although it is generally accepted that the serotonin-containing granules of cells are distinct from mitochondria (Green *et al.*, 1962), the presence of monoamine oxidase in the latter suggests a relationship of serotonin to mitochondria in functioning cells.

It is possible that the serotonin-induced swelling is mediated through functional changes of the electron transfer coupled with phosphorylation. The inhibitory effect of succinate against the serotonin-induced swelling may be explained by the energetically driven electron backflow in the NAD-branch (Chance and Hollunger, 1961) or by the competition of succinate for oxidation through the flavoprotein-cytochrome system (Krebs *et al.*, 1961), either of which prevents the forward flow of electrons in the NAD-branch of the respiratory chain. The finding that succinate actively inhibited β-hydroxybutyrate-induced swelling at the higher concentration of the inducer rather than at the lower one favor the former explanation. Some specificity of the linkage between β-hydroxybutyric dehydrogenase and the respiratory chain was described

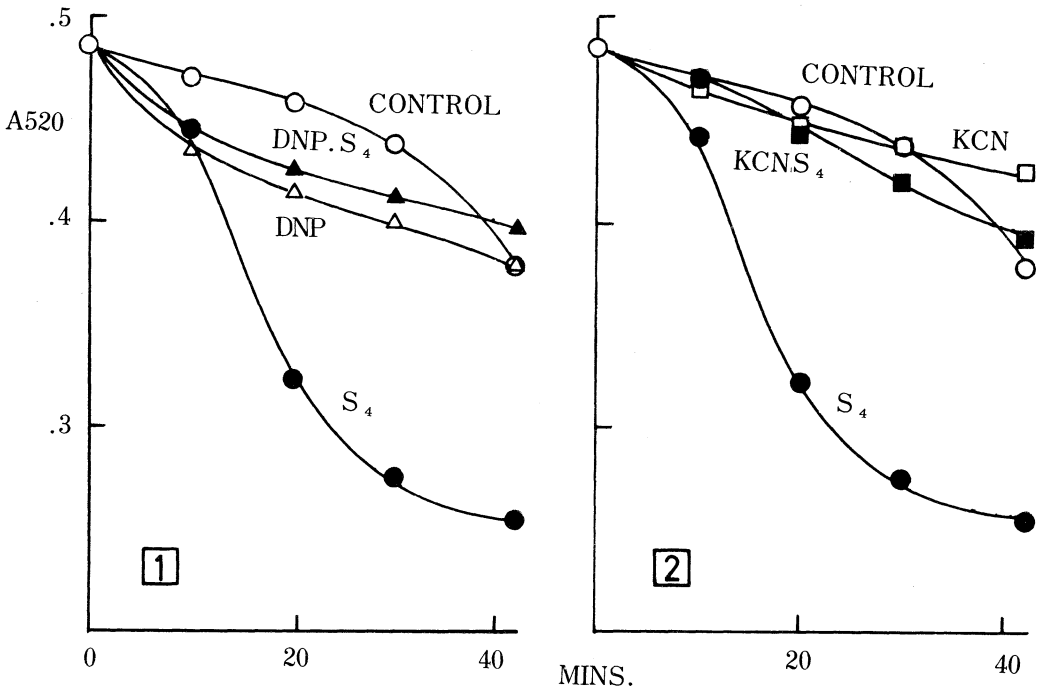

Fig. 15. Prevention of serotonin-induced swelling by respiratory chain modifiers. Serotonin ($5 \times 10^{-4}M$; S_4), when present, added last at time zero. Other additions were, 1. dinitrophenol (DNP) $1 \times 10^{-4}M$, 2. potassium cyanide (KCN) $1 \times 10^{-3}M$. Test medium was $0.125M$ KCl-$0.02M$ Tris-HCl, pH 7.4.

by Wise and Lehninger (1962). It has been found separately that the oxygen consumption of mitochondria with succinate as substrate is inhibited by serotonin only slightly in comparison with those with NAD-linked substrates. The acceleration of succinate-induced swelling by amytal can be interpreted as the result of a blocking of electron back flow into the NAD-branch and an increase in forward flow into cytochrome systems. The discrepancies between the present findings and those of Hunter *et al.* (1959) on the action of succinate and malonate may be ascribed to a possible difference in the integrity of the mitochondrial preparations employed. In fact, when mitochondria was prepared by the use of deionized, quarz-redistilled water in this laboratory, the resulting mitochondria were swollen definitely by succinate and not by α-ketoglutarate.

The preventive action of serum albumin against the serotonin-induced swelling may be taken to show that, as discussed by Wojtczak and Lehninger (1962), the swelling is mediated through the endogenous accumulation of free fatty acids. Also likely is a possibility that albumin facilitates the reduction of intramitochondrial NAD, as proposed by Klingenberg (1961), to stabilize the mitochondria against deterioration (Kaufman and Kaplan, 1959). On the other hand Kerp and Kasemir (1962) reported the quantitative combination of serotonin with human serum albumin. However, the nearly complete inhibition of the swelling elicited by albumin can not be accounted for by the 40 per cent decrease, at most, of the available serotonin in the medium. Preliminary experiments in this laboratory using colorimetric microdetermination have shown that fatty acids actually accumulate during the course of serotonin-induced swelling. Whether the fatty acid accumulation is the primary cause or a result of serotonin action awaits further studies.

Serotonin has been known to be an inhibitor of lipid peroxidation (Bernheim *et al.*, 1957). The present finding that ascorbate-induced lysis was prevented by serotonin while the serotonin-induced swelling occurred practically in a normal fashion may be explained by the inhibition of lipid peroxide formation. A similar phenomenon has been reported by Hunter *et al.* (1963) on the interaction of phosphate and glutathione-induced swelling. Serotonin-induced swelling can safely be distinguished from the lytic phenomena caused by ascorbate, ferrous ion, or glutathione.

It is probable that serotonin interacts with some components of the NAD-branch in the respiratory chain, causing changes of oxidation-reduction state of the carriers. Serotonin is not only an electron donor (Szent-Györgyi, 1960; Cilento and Tedeschi, 1961) but also may form charge-transfer complexes in the oxidative phosphorylation mechanism as proposed by Kosower (1962). Iodine and iodine cyanide, whose charge-transfer complexes with pyridine and flavin derivatives have been well documented, are known to induce electron transport-supported mitochondrial swelling (Rall *et al.*, 1962, 1963). It seems possible that the mechanism of action of these iodine-containing compounds in mitochondria has relevance to that of thyroxine. Furthermore, some investigators, as represented by Woolley (1962), believe that serotonin has a primary role in the transportation of Ca^{2+} in various tissues. Serotonin action has been ascribed by Woolley to the formation of complex with Ca^{2+} (Woolley and Campbell, 1960). Since it has been reported that swelling induced by Ca^{2+} is mediated through the U-factor (Wojtczak and Lehninger, 1962), a triple interaction of serotonin, Ca^{2+} and U-factor is by no means unlikely.

These considerations suggest that strictly comparable study of serotonin, thyroxine and parathormone on mitochondrial morphology and function may be fruitful for the clarification of the mode of action of these agents,

and also for the elucidation of a possible interaction between glandular hormones and the so-called tissue hormones. Calcitonin as well as melatonin should also be interrelated in this endeavour. It may also be valuable to study morphological and functional alterrations of the liver mitochondria from malignant carcinoid patients, who show hyperserotonemia.

Quite recently, effects of serotonin on functional state of mitochondria from heart (Rizzoli, 1967; Zubovskaya, 1968) as well as brain (Mahler and Humoller, 1968) were reported. These reports seem to relate the mitochondrial effect to the pharmacological activity in tissues.

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