Reports

Biphasic Action of Reserpine and Isocarboxazid on Behavior and Serotonin Metabolism

Abstract. In longitudal studies on psychiatric patients receiving therapeutic doses of reserpine and isocarboxazid, singly or in combination, we observed that the aggravation of psychotic symptoms sometimes occurring during treatment with reserpine was associated with a marked rise in urinary 5-hydroxyindoleacetic acid. Tranquilization was accompanied by a more moderate increase under the influence of reserpine, or by a reduction of 5-hydroxyindoleacetic acid excretion while on monoamineoxidase inhibitor medication. It is concluded that a moderate rise of free serotonin in the brain is associated with tranquilization, while a greater increase is accompanied by excitation.

It has been postulated that the psychic alterations induced by reserpine and monoamineoxidase inhibitors are mediated by biogenic amines such as catecholamines and serotonin (*1*). Most of the data on which this postulate is based were obtained in animal experiments by using relatively large doses of drugs. It is the purpose of this study to show whether or not correlations between behavioral changes and serotonin metabolism can be established in psychiatric patients receiving therapeutic doses of these psychotropic drugs.

Twelve patients, nine with schizophrenia (patients A to I inclusive) and three with mental deficiency (patients K to M inclusive), were placed on a constant protein diet previous to and during the entire period of investigation which averaged 47 days. They were given daily examinations including psychiatric interviews. The daily urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) was determined according to the method of Udenfriend *et al.* (*2*), and the 5-day averages were calculated on a basis of 100 g of protein per day. The investigation was divided into four periods: (i) placebo I; (ii) medication I in which seven patients received reserpine (Serpasil, Ciba, 4 mg/day), while isocarboxazid (Marplan, Hoffmann-LaRoche, 30 mg/day) was given to the other five patients; (iii) medication II in which all patients were placed on combined medication (Serpasil, 4 mg/day; Marplan, 30 mg/day); and (iv) placebo II. All medications were dissolved in water and taken orally.

We observed an aggravation of psychosis in six of the schizophrenic patients (Fig. 1). In three, (B, E, and G) the worsening occurred during the reserpine period, and two of these (B and G) suffered a second aggravation of the psychosis shortly after withdrawal of both drugs. In another schizophrenic (C) the first clinical symptoms of an activation of psychosis were observed towards the end of the combined medication, culminating in a severe state of mental disturbance after cessation of the isocarboxazid-reserpine treatment. In addition two patients (A, D) became mentally disturbed only in the second placebo period. Thus an activation of the psychosis was noted in 5 of 9 schizophrenics during the final placebo period. These changes in behavior were always accompanied by marked rises in urinary 5-HIAA in comparison with control levels. In addition evaluations of the daily changes showed increases in urinary 5-HIAA excretion previous to the activation of the psychosis. Clinical improvement was always associated with a fall in urinary 5-HIAA toward or to control levels.

Tranquilization took place in most patients during each of the three periods: with isocarboxazid, isocarboxazid-reserpine, and the second placebo period. We did not observe tranquilization during the initial reserpine medication, probably because its duration was too short to permit the calming action of that drug to develop. While under the treatment with the monoamineoxidase inhibitor, the most marked tranquilization was noted when 5-HIAA had fallen to its lowest values (left side of Fig. 2). This correlation was seen in 9 of 12 patients (C–H, K–M), while three others did did not become tranquilized. Two of the latter (A and I) showed only minor changes in behavior, although a reduction of urinary 5-HIAA was evident. Patient B, whose psychosis was activated during the reserpine period, improved somewhat under reseprine-isocarboxazid treatment and subsequently became much worse following the withdrawal of the combined medication. During disturbed periods his urinary 5-HIAA was always above control levels.

In those patients who retained tranquilization or became tranquilized during the second placebo period (right side of Fig. 2), we observed either reduced outputs of urinary 5-HIAA when monoamineoxidase inhibition was still retained as indicated by persistent high tryptamine levels, or merely moderate increases of that serotonin metabolite, when presumably a residual effect of reserpine continued for sometime after that of isocarboxazid had ceased (F, M and E, H, I, K, respectively).

Our observations were made on mental patients receiving much smaller doses of reserpine and isocarboxazid than the doses used in animal experiments. The correlations between behavioral changes and urinary excretion of 5-hydroxyindoleacetic acid indicate that, under our experimental condition, tranquilization was accompanied by a moderate increase of free serotonin in the body, while a greater rise was found previous to and during the aggravation of the psychosis in our schizophrenic patients. The clinical symptomatology of the acute exacerbations always followed the same pattern as in the recurrent activations the patients had previously experienced without any medication. An activation of the psychosis did not occur in 4 out of 9 schizophrenic patients and we never observed a psychosis-like state or marked excitement in the mentally defective group. In these patients, the urinary 5-HIAA excretion did not attain the elevated levels found in the other five schizophrenics previous to and during the periods of mental disturbance. From our results it seems that the endogenous indole metabolism of those schizophrenic patients who became mentally disturbed was more labile, for the relatively low doses of the drugs employed induced large alterations in serotonin turnover.

Figure 3 may be regarded as a schematic representation of our results

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

as well as a theoretical exposition of the drug effects in terms of free and bound serotonin. Placebo I represents the initial metabolic situation with more bound than free serotonin. Reserpine (medication Ia) releases serotonin from its bound form, thus producing a large rise in free serotonin which was found to be associated with an aggravation of the psychosis. A later effect (medication Ib) suggests a subsequent result of reserpine after the bulk of free serotonin has been washed out. Free serotonin is still increased, but the actual concentration is increased to a lesser extent depending on its rate of formation and destruction. This moderate rise is associated with tranquilization. It would seem that, when the serotonin concentration was low previous to reserpine medication, only a moderate rise of free serotonin could be evoked by reserpine, which again is also associated with tranquilization.

The period in which the patients received only isocarboxazid is omitted in this figure, as the behavioral and metabolic effects were essentially the same as those occurring during the combined isocarboxazid-reserpine medication. It would seem that the monoamineoxidase inhibitor antagonized the action of reserpine to release bound serotonin.

Giarman and Schanberg (*3*) came to a similar conclusion as they found that iproniazid alone, as well as in combination with reserpine, raises both the bound and free forms of serotonin in the brain, and the latter form to a greater extent. Probably the rise of free serotonin becomes most pronounced after saturation of the binding sites is achieved.

Our results suggest that a moderate increase of free serotonin during isocarboxazid-reserpine medication is correlated to tranquilization (medication IIa) while a greater increase (medication IIb) is associated with excitement.

The final placebo period (placebo II) is characterized by differential rates of decline in the activities of reserpine and isocarboxazid. There are individual differences, but as a rule reserpine effect is retained longer than the effect of the shorter-acting isocarboxazid. Another factor to be considered is the magnitude of the serotonin stores achieved just before drug therapy had been discontinued. As examples of a great number of possible results on serotonin and behavior after cessation of the combined medication, five different situations are outlined. If serotonin was moderately elevated during the reserpine-isocarboxazid period (medication IIa), and if isocarboxazid remains predominant at the beginning of the second placebo period, no additional serotonin can be re-

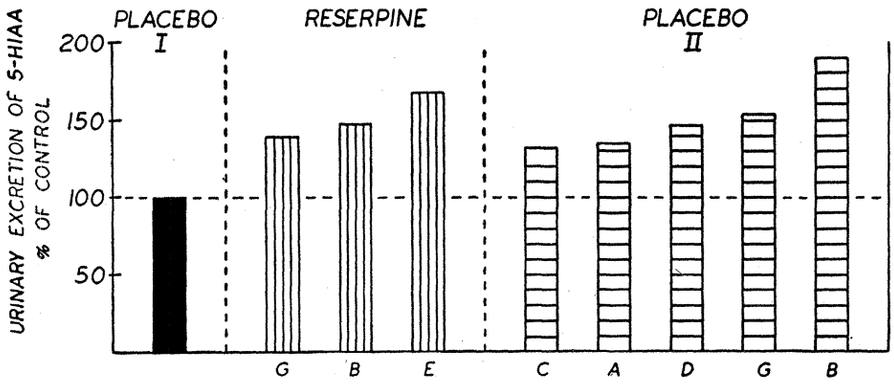

Fig. 1. Urinary excretion of 5-hydroxyindoleacetic acid during mentally disturbed periods. The bars represent the averages of 5-day periods in which the state of mental disturbance was most pronounced.

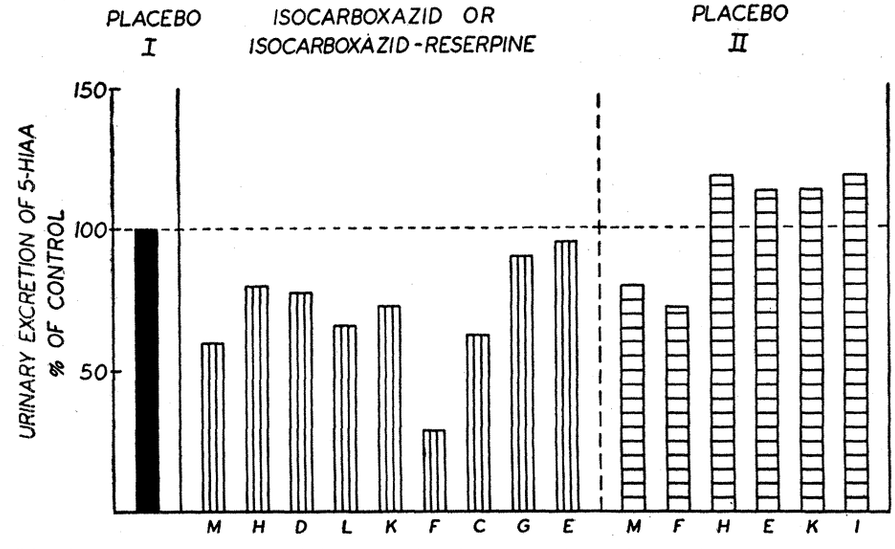

Fig. 2. Urinary excretion of 5-hydroxyindoleacetic acid during periods of tranquilization. The bars represent the averages of 5-day periods in which tranquilization was most marked.

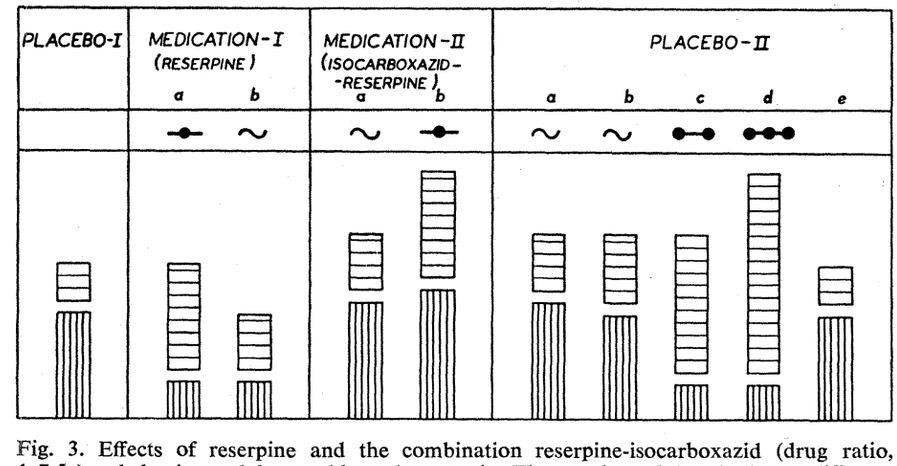

Fig. 3. Effects of reserpine and the combination reserpine-isocarboxazid (drug ratio, 1:7.5)on behavior and free and bound serotonin. The number of dots indicates different grades of excitement and mental disturbance; the wavy lines stand for tranquilization. The vertically lined bars represent the bound form of serotonin, and the horizontally lined bars represent the free form of serotonin.

leased and tranquilization is retained (placebo IIa). If, however, the isocarboxazid effect is weakened and merely a small amount of serotonin is released by the surviving action of reserpine, tranquilization continues as a behavioral correlate (placebo IIb). But when the reserpine effect is predominant over the isocarboxazid effect, and when most of the previously stored serotonin is released, free serotonin rises to the high levels associated with severe mental disturbance (placebo IIc). If the serotonin level was elevated markedly during the reserpine-isocarboxazid medication as indicated under medication IIb, and if reserpine continues to act after the monoamineoxidase inhibitor effect has ceased, even greater amounts of serotonin can be released and grave mental disturbance is observed (placebo IId). Finally, when both drugs have ceased to be effective the original biochemical and behavioral states are restored (placebo IIe).

The results show a close correlation between drug-induced alterations of behavior and serotonin metabolism, and they point to the conclusion that it is the free form of serotonin which parallels the behavioral changes. We know from clinical observations that both drugs, reserpine and monoamineoxidase inhibitors, may exert biphasic effects on the behavior of mental patients. They can evoke in the patient tranquilization as well as behavioral disturbance and aggravation of psychosis. These observations have their counterparts in the pharmacological effects of both drugs on serotonin metabolism; for both drugs, though acting by different mechanisms, can elevate free serotonin in the body, and it depends on the extent of this elevation whether tranquilization or disturbance will appear as a behavioral correlate. Whether or not the observed changes of indole metabolism also apply to the brain has not been determined in the present study. Experiments on animals have shown, however, that small elevations of free serotonin in the brain evoke a sedative effect, while larger elevations produced by high doses of 5-hydroxytryptophan, with or without monoamineoxidase inhibitors, induce excitement and disturbed behavior (*1, 4*). Further studies are in progress to verify the results presented here on a greater number of patients and also to determine biochemical parameters other than serotonin, as the psychotropic drugs studied affect the metabolism of many chemical constituents of the body.

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Variation of Enhancement of Photosynthesis with Conditions of Algal Growth

Abstract. The Emerson effect was observed as an enhancement of photosynthesis in long-wavelength red light when beams of shorter wavelength were added. Two light beams of wavelengths 650 and 694 mμ, respectively, when presented together, gave a photosynthetic rate higher than the sum of the rates obtained separately. The amount of enhancement of photosynthesis depends upon the growth conditions of algal cells, and specifically on their phosphate metabolism.

Emerson discovered that photosynthesis at a wavelength of about 700 mμ, which is usually inefficient in green plants, becomes more efficient when light beams of shorter wavelength are presented simultaneously (*1*). This he called enhancement. The enhancement of photosynthesis may be defined as the ratio of the rate of photosynthesis obtained by simultaneous illumination with beams of two wavelengths to the sum of the photosynthetic rates produced by illumination with each wavelength separately. The action spectrum of the effect caused by beams of shorter wavelength has been measured in several laboratories. Chlorophyll *b* is the chief pigment causing better utilization of the long-wavelength light in green algae (*2*). Furthermore, the same function is carried out by the phycobilins in red and blue-green algae (*3, 4*). In different organisms enhancement may be variously achieved by the simultaneous activation of certain forms of chlorophyll *a* and of an accessory pigment which may be either chlorophyll *b*, the 673-mμ form of chlorophyll *a*, a phycobilin, or a carotenoid (*4*).

In a further analysis of enhancement, the effects of culture conditions have been examined. In this report the consequences of varying the age and the nutrition of *Chlorella vulgaris* and *C. pyrenoidosa* are discussed.

The unicellular algae *C. vulgaris* and *C. pyrenoidosa* were cultured in Knops solution of the following composition: $MgSO_4 \cdot 7H_2O$, 0.0103*M*; KNO_3, 0.0124*M*; KH_2PO_4, 0.0092*M*; K_2HPO_4, 0.0092*M*; and 1 ml of trace element mixture (*5*). Air containing 5 percent CO_2 was bubbled through the culture during growth. Continuous illumination was provided by two 15-watt fluorescent lamps 15 cm from the culture tubes (light intensity about 300 ft-ca). The temperature was held at 23.4°C.

Photosynthesis was measured by a polarographic method in which a stationary bright platinum electrode was used (*6*). The cells were illuminated by two light beams. The first beam, of wavelength 650 mμ, was presented by a monochromator with slits adjusted to give half band width of 5 mμ. A second light beam, of wavelength 694 mμ, from a small slide projector, presented a slightly oblique illumination to the cells. Its intensity was controlled by the lamp voltage, and its spectral character, by an interference filter having a half band width of 11 mμ, centered at 694 mμ. Red glass was used to cut off wavelengths shorter than 660 mμ.

The projector beam was adjusted to an intensity such that, after switching from the monochromator beam, an equal steady-state photosynthesis rate was obtained. The monochromatic beam was then added to the projector beam for the time necessary to obtain a new steady rate of photosynthesis. The procedure gives enhancement in terms of the total light presented—namely, as

$$\text{Enhancement} = \frac{\text{rates } (650 + 694\ \text{m}\mu) \text{ presented together}}{\text{rate } 650\ \text{m}\mu + \text{rate } 694\ \text{m}\mu \text{ presented separately}}$$

Several algal species (*Ochromonas sp., Navicula minima, Amphora exigua*) cultured in this laboratory on complete media so far have never shown bichromatic enhancement of photosynthesis, perhaps because of unfavorable culture conditions or age. Table 1, lines 1 to 3, gives illustrative data.

Enhancement has always been found under favorable conditions in *Chlorella vulgaris* and *C. pyrenoidosa*. In these organisms the enhancement of photosynthesis varies during the growth cycle of algal cells. It is at a maximum in the logarithmic phase of growth, decreases during the stationary phase, and be-

Table 1. Variation of enhancement with conditions of culture.

Organism	Enhancement value
Ochromonas sp.	1.0
Navicula minima	1.0
Amphora exigua	1.0
A. exigua with pyrophosphate	1.5–1.6
C. vulgaris and *C. pyrenoidosa*, log phase	1.5–1.79
C. vulgaris and *C. pyrenoidosa*, stationary phase	1.3–1.5
C. vulgaris and *C. pyrenoidosa*, aged cells	1.0–1.1