INTERACTIONS OF RESERPINE, SEROTONIN AND RELATED DRUGS, AS INDICATED BY ENERGY METABOLISM, THYROID AND ADRENAL FUNCTION OF THE RAT

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Ranbir Krishna Bhatnagar

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By

Ranbir Krishna Bhatnagar

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Still we persist, plow the light sand and sow seed after seed, where none will ever grow. - JUVENAL.

Dedicated
to the
'Unknown'

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INTRODUCTION

There is continuously growing evidence involving the field of hypothalamo-hypophyseal physiology with the integrative functions of the central nervous system. The research centered around such integrative and regulatory systems, has embraced the disciplines of biochemistry, anatomy, neuropharmacology, psychopharmacology, neuro-endocrine mechanisms and many others. Recently much interest has been focused on the use of certain pharmacological agents not only to understand their pharmacological effects but also to get an insight into some of the neuroendocrine mechanisms. These agents are either directly involved in many of the physiological processes, induce the release or alteration of substances present in the central nervous system or act peripherally.

A number of pharmacologically active substances naturally occurring in the central nervous system have been studied. It is believed that these agents are neurohumoral substances stored inside the cell in the mitochondria or surrounding it, protected by intracellular barriers from various enzymes which are also present in the cell, for the synthesis and biotransformation of these neurohumors. On release they become physiologically active and regulate neuronal transmission

or other functional processes. The concept of catecholamine release as a mechanism of drug action has been considerably expanded in the past few years and during the last decade serotonin has attracted more attention than any other pharmacologically active substance. The introduction of several new drugs in treatment of psychotic conditions has revived great interest in the chemical basis of these conditions.

The physiological significance of certain amines occurring in the hypothalamus is still obscure. These compounds have been conclusively shown to have certain pharmacological actions and have been proposed as neuroendocrine transmitters. The experiments to be reported were designed to determine whether they are directly involved in the central autonomic control of certain of the endocrine glands.

Reserpine is known to cause the release of serotonin and catecholamines, accompanied by certain mental and endocrine disturbances. The latter two amines are present in localized areas in the central nervous system. They are strongly believed to have some role in neuronal transmission there.

A comparison of the acute and chronic effects of reserpine and serotonin on adrenal weights, thyroid activity, and energy metabolism was undertaken in an effort to answer the questions whether 1) the serotonin released by reserpine is the main

contributing factor for the manifestation of reserpine actions? 2) are these actions due to the depletion of serotonin stores? 3) are reserpine actions mediated by one or more other factors?

To elucidate these questions it was of importance to study the serotonin-monoamine oxidase system. To further facilitate the study, 5-HTP the precurser of serotonin and BOL, a specific serotonin antagonist, was used in various combinations with reserpine and serotonin.

REVIEW OF LITERATURE

The rapid expansion of research in both the fields of pharmacology and physiology has made it increasingly difficult to keep abreast of the literature even in the field of one's own interest. More so since pharmacology is intimately allied to other disciplines of the biological sciences, the author has tried to be selective and much important work has inevitably been ignored hoping that the omissions would not lead to serious misrepresentations of the neurochemical, neuroendocrine and neuropharmacologic views that have been advanced to date.

I. Neural Control of Anterior Pituitary Functions

During the past decade newer concepts of an infinitely variable nature in the fields of endocrinology, neuropharmacology and neuroendocrine mechanisms have emerged. The influence of psychic states and exteroceptive stimuli on the hormonal control has clearly shown the neural influence on the adenohypophysis. Secretomotor nerve fibers are absent in the adenohypophysis (Rasmussen, 1938). The hypophyseal portal blood system first described by Popa and Fielding (1930) is known to drain blood from the median eminence and

the stem into sinusoids of the pars distalis (Wislocki and King, 1936, Green and Harris, 1949). Earlier workers (Green and Harris, 1947) explained the relationship between the central nervous system and endocrine glands through this portal system--the only accepted vascular connection between brain and pars distalis. Severance of vascular connections between the hypothalamus and the adenohypophysis caused a marked reduction in ACTH, TSH, FSH, LH, and STH secretion (Harris, 1955). This suggested that the vascular supply is essential for the normal functions of the anterior pituitary. Harris (1955) proposed the probability of the hypothalamus being most directly concerned with the regulation of the adenohypophysis. According to this concept, the neurosecretory material is discharged under the influence of exteroceptive or visceral impulses and is transported through the hypophyseal portal system to the pars distalis to induce the release of hormones. The morphological arrangement in the median eminence and infundibular stem favors the view that neurosecretory material may serve as a neurohumoral link between the central nervous system and the anterior pituitary.

Mammals have a neurosecretory mechanism in the hypothalamo-hypophyseal system (Harris 1955). The neurosecretory material appears in the neurons and cell processes of the

supraoptic and para ventricular nuclei. These granules lie in the axons which comprise hypothalamo-hypophyseal tract from their origin in the two nuclei to their termination on the vessels of the infundibular process. Palay (1953) presented a hypothesis that neurosecretory material may serve as the hormonal link between the hypothalamus and the adenohypophysis.

Electrical stimulation of the hypothalamus induced the secretion of adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH) and gonadotropins (FSH-LH) (Harris, 1955). Discrete lesions were placed in the hypothalamus to show the impairment of ACTH (de Groot and Harris, 1950) and TSH (Greer, 1952) secretion. This study indicated the localization of centers for each tropic hormone in the hypothalamus.

The nature of hypothalamic neurohumoral agents involved in the anterior pituitary functions is not clear. However, ACTH secretion is evoked by a corticotropin releasing factor (CRF). This factor has been recently isolated from the hypothalamus (Guillemin, 1962). A hypophyseal LH-releasing factor resides in the stalk-median eminence tissue (McCann 1962). Evidence has accumulated for the presence of a thyrotropin releasing factor (TRF) (Shibusawa, 1956). PIF-prolactin inhibiting factor (Talwalker et al., 1963) and a growth hormone

stimulation factor (Deuben and Meites, 1963). CRF and TRF are apparently polypeptides (Schreiber et al., 1963). Thus the hypothalamus can be considered as the final common pathway for environmental and, therefore, neural influence on the anterior pituitary.

A certain antagonism can be demonstrated between the autonomic and neuroendocrine functions of the anterior and posterior hypothalamic zones. Reciprocal inhibition of the anterior and posterior hypothalamic zones has been shown in cats. Two reciprocally inhibitory systems concerned with autonomic and endocrine functions coexist in the brain, and they are represented in, but cannot be too specifically localized within the hypothalamus. The more caudal end of these two systems may be found in the midbrain with evidence of the presence of facilitory and inhibitory areas for pituitary-adrenal response. Thus there is an indication that at the hypothalamic site these facilitory and inhibitory areas are reciprocally innervated so that the activities of one inhibit the activities of others (Bovard, 1961).

TRF has been found in blood, cerebrospinal fluid, urine and posterior pituitary in high concentrations (Shibusawa, 1959). Schreiber et al., (1963) have shown the evidence for TRF in hypothalamic extracts. However, it has been reported

that TRF of Shibusawa has no thyrotropin releasing activity (Reichlin, 1963). As far as TSH release is concerned, accumulated evidence suggests that the anterior basal part of the hypothalamus is essential for maintenance of TSH release (Bogdanove, 1953; Greer, 1956; Florsheim, 1958; D'Angelo, 1958).

Despite the accumulated information and almost universal agreement that the brain must play an important role in the regulation of TSH secretion the nature and extent of this role is not clear (Bogdanove, 1962).

II. Reserpine

A. General Pharmacological Actions

Extracts from Rauwolfia serpentina, a small shrub indigenous to certain parts of India have been employed in Ayurvedic medicine for centuries (Moore et al., 1954). Muller et al. (1952) isolated a crystalline alkaloid (reserpine, serpasil) from Rauwolfia serpentina. Since then the structure of reserpine has been identified and synthesized. This produced a prolonged CNS depression in laboratory animals and a fall in blood pressure of anaesthetized cats and rabbits (Bein, 1953). Bein also indicated the possibility of a central origin of serpasil-induced hypotension. Evidence indicating a central site of action for serpasil has ben cited (Trapold

et al., 1954). They postulated that serpasil produces a central inhibition of the sympathetic nervous system, possibly through specific hypothalamic depression. Since then, the pharmacology of Rauwolfia alkaloids including reserpine has been studied by many workers (Plummer et al., 1953, 1954). Schneider (1954) observed a drop in body temperature, an equivocal drop of blood pressure and a reduction of heart rate and respiration rate. He proposed the main point of attack of the drug to be in the brain stem.

Bein (1953) and Bein et al., (1953) concluded that serpasil acts directly on the central autonomic system, possibly in the hypothalamus.

Hess (1947) showed that the stimulation of certain nuclei in the supra optic area of the hypothalamus of the cat produced a peculiar state of quiescence or sleep, associated with miosis, lowering of body temperature, fall of blood pressure, bradycardia, increased intestinal activity and slowing of respiration. Based on their experimental evidence Bein et al. (1953) suggested that rather than a stimulation of these parasympathetic centres reserpine caused a depression of the corresponding central sympathetic functions. Tripod and co-workers (1954) and Schneider and Earl (1954) stated that reserpine-induced quiescence is distinctly different from

sedation induced by barbiturates. These further clinical and experimental findings led to the assumption that the final result of the reserpine effect is a depression of central sympathetic structures of the diencephalon. However, the exact mode of action is not clear.

Sham rage in cats was counteracted by reserpine (Schneider, 1955). The blood pressure rise after direct electrical stimulation of the diencephalon in anaesthetized cats was not decreased after reserpine, whereas the carotid occlusion reflex was lessened. Taking support from his own and previous work Schneider (1955) postulated that reserpine causes a central block or inhibition of afferent impulses which normally stimulate the sympathetic activity rather than a direct depression of diencephalic sympathetic centres.

Himwich and Rinaldi in their personal communication to Schneider and co-workers (1955) were the first to point out the stimulatory effect of reserpine on the reticular formation of the brain stem. Clinical findings during this period by Chen and co-workers (1954) and others, led to the belief that reserpine might have a far more general influence on the CNS than originally assumed. Chen (1954) thought that reserpine causes an increase in the excitability or in the conductivity of certain CNS structures. Schneider and co-

workers (1955) demonstrated a facilitory action of reserpine on synaptic transmission and speculated about the possible involvement of an acetylcholine-like mechanism.

The responses of the CNS and blood pressure (BP) to reserpine are slow in onset and, therefore, possibly reflect an action not initiated by the reserpine molecule *per se*. Attempts were made to correlate these reserpine actions to serotonin, an indole amine, which was shown to be released by reserpine from brain, platelets and intestine (Pletscher and co-workers, 1955, 1956).

Maxwell (1957), studying its peripheral action, found that reserpine produced pressor responses in the spinal dog, contrary to what was believed. This occurred within a few minutes and was completed in a short time. They indicate it to be peripherally evoked and suggested the involvement of a sympathomimetic humoral mechanism.

Serotonin administered to mice produced sedation and potentiated hexobarbital hypnosis by a central action, as does reserpine (Shore and co-workers, 1955a).

Reserpine administered to dogs induced a marked increase in the urinary excretion of 5-hydroxyindole acetic acid (Shore *et al.*, 1955b). The latter was found to be a major metabolite of serotonin (Titus and Udenfriend, 1954). In

rabbits reserpine liberated serotonin from its major body depot, the intestinal tract (Pletscher et al., 1955).

On the basis of these and their own results Pletscher and co-workers (1956) postulated that serotonin is important in normal brain function and that its release from the cells is mediated by reserpine. Maickel (1961) reported that treatment of rats with reserpine produced a biochemical picture almost indistinguishable from the classical stress response evoked by cold. These effects were attributed to the release of ACTH as they were absent in hypophysectomized and adrenalectomized rats. Recently Westermann and co-workers (1962) have shown a close association between the ACTH hypersecretion, decline in content of brain monoamine stores and the central activity produced by reserpine. They showed that the ACTH discharge is not related to the change in stored norepinephrine and that the central activity of reserpine is related to the blockade of serotonin storage and not to that of norepinephrine. Hence it was postulated that the hypersecretion of ACTH results from the action of reserpine on neuronal pathways that monitor the anterior pituitary. Costa et al. (1962) (Cited by Westermann et al., 1962) have shown that the action of reserpine on the pituitary is part and parcel of its sustained action on neuronal pathways in the brain.

Despite a considerable literature on a possible biochemical explanation of reserpine action such mechanisms have not been localized in the brain to a specific area such as the reticular formation. Pletscher and co-workers (1956) have reported a greater concentration of serotonin in the brain stem than in the remainder of the brain and therefore a greater absolute quantity released after injection of 250 μg of reserpine in the rabbit. Brodie and co-workers seem convinced that release of serotonin results from an inability of tissue to bind certain amines and is the basis of the sedative effects of the Rauwolfia alkaloids. However, the data thus far are not conclusive that such selective effects operate in the brain stem or even how important the serotonin level is to brain activity (Killam, 1962).

B. Effect on Adrenal Activity:

By now it became apparent that reserpine treatment affects the endocrine system. With the accumulated evidence that both monoamines and catecholamines are released by reserpine, both centrally and peripherally, much attention was drawn to the assumption that some neurohumoral mechanism may be involved in the action of reserpine.

Effect of reserpine simulating stress was believed to be due to the involvement of ACTH. Attention was drawn to the

pituitary-adrenal axis in response to reserpine and stress. Several workers demonstrated that large doses of reserpine deplete most of the ACTH stores in the hypophysis (Kitay et al., 1959; Saffran and Vogt, 1960). Saffran and Vogt (1960) reached the conclusion that the release of ACTH after reserpine could be explained entirely on the basis of its stressing action. They found that reserpine-depleted stores of 5HT, take many days to fill up again in comparison to nitrogen mustard, a highly toxic substance, which causes a great loss of ACTH and an effect of shorter duration. Since formation of 5HT is a very fast process (Brodie et al., 1958, cited by Saffran and Vogt, 1960) the delay can only be accounted for by a prolonged action of reserpine.

Mason and Brady (1956), Mahfouz and Ezz (1958) and Wells et al. (1956) observed that reserpine inhibited the response of the rat to acute stress as measured by the degree of adrenal ascorbic acid depletion. It was postulated that reserpine depresses the response of the rat to acute stress by inhibiting the hypothalamic centres (Sevy et al., 1957).

Mahfouz and Ezz suggested an action of the prepituitary phase of response to stress, possibly on the hypothalamus. It has been sugested that adrenal hypertrophy and ACTH hypersecretion occur during reserpine treatment (Gaunt et al., 1954, Hertting et al., 1957, and Tindall, 1959).

Reserpine in a single dose of 2.5 mg/Kg, in rats, raised the plasma and adrenal corticosterone and caused an increase in the weight of adrenal glands (Montanari and Stockham, 1962). These authors assumed these changes as a consequence of persistent and prolonged ACTH discharge from the pituitary as they were absent in hypophysectomized animals. They further showed in contrast to Maickel et al., (1961) that whether or not ACTH depletion occurs as a result of reserpine treatment there is still enough to be released in response to acute stimulus. Their results also indicate that reserpine causes ACTH release only when given in doses which induce sedation and loss in body weight. 0.1 mg/Kg for ten days produced no overt sedation and loss in body weight and there was no increase in the basal plasma corticosterone level or adrenal gland weight. However, 0.5 mg/Kg for 5 or 9 days and 2.5 mg/Kg for 6 days induced marked sedation and loss in body weight together with a raised plasma corticosterone and an enlarged adrenal gland. A similar enlargement in adrenal gland has been reported (Gaunt et al., 1954; Hertting et al., 1957; Kitay et al., 1959).

These results suggest that whatever depletion of ACTH stores in the pituitary has occurred as a result of prolonged reserpine treatment, it is possible to obtain the release of additional ACTH in response to an acute stimulus such as ether.

On the contrary, Wells (1956) and Kitay et al. (1959) found an inhibition of pituitary ACTH release after administration of reserpine. Christian (1955) observed a suppression of adrenal hypertrophy in male mice. Tindall (1959) reported thymus atrophy in rabbits indicating pituitary-adrenal cortex hyperactivity during reserpine administration.

Khazan (1961) showed through acute pretreatment that reserpine has a stimulating effect on the pituitary-adrenal cortex as revealed by adrenal hypertrophy, adrenal ascorbic acid depletion and increased 17-KS and 17-OHCS levels. They called it a non specific stimulus which has a maximum effect on the pituitary-adrenal axis, an effect which disappeared during chronic treatment.

In addition, the work of Kitay et al. (1959), Westermann et al. (1960) Saffran and Vogt (1960) indicated that reserpine decreases the content of ACTH in the rat pituitary.

Gaunt et al. (1961) point out that reserpine and other drugs first evoke an outpouring of ACTH, but later the stimulatory action subsides and the drugs now counteract the ACTH releasing effect of other stimuli. Wells et al., (1956) postulated that reserpine, like morphine, first acts as a stressful stimulus and then causes an adaption of the pituitary to the drug stress.

Munson (1961) considers that initial hypersecretion of ACTH comes to an end because the pituitary becomes adapted and the stimulating action of reserpine on the pituitary is then replaced by inhibition.

In view of these conflicting reports Maickel and Co-workers (1961) indicated that both reserpine and cold exposure can elicit hypersecretion of ACTH. Large doses of reserpine or prolonged cold exposure lowers the pituitary content of ACTH to such an extent that the animals are unable to respond to an additional pituitary stimulus. There is 75% depletion of ACTH within 20 hours of a single injection of reserpine and further injection of reserpine does not change ascorbic acid content of adrenals or plasma corticoids, suggesting that the rate of ACTH discharge is faster than synthesis.

Westermann and co-workers (1962) noted that adrenal hypertrophy and increased activity of liver Tryptophane pyrrolase (TPO) are measures of the cumulative effect of ACTH. These indices, they observed, are particularly useful in describing a prolonged pituitary stimulation as a high TPO activity as well as adrenal hypertrophy can persist long after levels of corticosterone have returned almost to normal.

Maickel et al. (1961), Westermann et al. (1962) and Montanari and Stockham (1962) have been able to clarify to some extent the conflicting views on the pituitary-adrenal axis in response to reserpine treatment. They suggest that ACTH secretion is controlled by the hypothalamic fibers that liberate a humoral substance into the hypohphyseal portal circulation. They further suggest that perhaps the secretion of this humoral agent is controlled by both inhibitory and stimulatory pathways in the hypothalamus as suggested by Egdahl (1960). Thus ACTH discharge might result from activation of stimulatory pathways or depression of inhibitory pathways. Reserpine might act in the latter way.

C. Effect on Thyroid Activity:

The introduction of reserpine as a therapeutic agent and its apparent involvement in neuronal pathways drew attention to its effects on endocrine function. Effects of reserpine and serotonin were studied on lactation (Meites, 1959). An antithyroid role has been ascribed to reserpine. Hyperthyroid patients treated with reserpine were observed to have a marked fall in Basal Metabolic Rate (BMR) (Pitt-Rivers and Tata, 1959). Kuschke et al., 1954, cited by Pitt-Rivers and Tata (1959). DeFelice et al. (1957) observed peripheral inhibition

of thyroxine action by reserpine in rats and guinea pigs, respectively.

Mayer and co-workers (1956) employed the thyroid slice I^{131} technique and found that reserpine in a concentration of 0.083 mg per ml of incubation medium inhibited organic binding of I^{131} by thyroid. Pokorny et al. (1957) showed that reserpine produced changes in cell heights and colloid content of calf thyroids similar to those produced by compounds which inhibit the synthesis of thyroxine.

Reserpine at doses of 5, 10, or 50 µg/100 g/day inhibited thyroid activity in rats (Moon and Turner, 1959). They found the thyroidal I^{131} output to be significantly decreased and thyroid secretion rate reduced approximately 86-90% below control. They suggested the alteration of thyroid function during reserpine treatment was through inhibition of thyrotropin secretion. Bierwagen and Smith (1959) studied the effect of reserpine on endogenous dehydrogenase enzyme activity of the thyroid and suggested that the mechanism of action for reduced enzyme activity was via the pituitary to produce a decreased TSH elaboration or release. Moon and Turner (1959a) blocked the endogenous TSH secretion with thyroxine and found that reserpine had no significant effect upon thyroidal I^{131} release resulting from injection of TSH.

They concluded the inhibition in thyroid function to be through suppression of TSH secretion.

Thus far it was suggested that reserpine may inhibit thyroid function by 1) acting as an antithyroid compound to inhibit the thyroidal uptake and organic binding of iodine; 2) reducing the secretion of TSH or; 3) acting as a thyroxine antagonist.

Thyrotoxicosis in human beings is believed to be caused by some diencephalic disturbances. Canary and co-workers (1957) reported that reserpine treatment in thyrotoxic patients caused a quick relief of symptoms and effects when given intramuscularly but the 24 hour I^{131} uptake and PBI did not change.

Yamazaki and co-workers (1961) determined the effect of reserpine in the mouse on the fractional rate of turnover of exogenous radiothyroxine and on the release of thyroidal radioiodine in response to exogenous thyrotropin and stable thyroxine. They confirmed the findings of Moon and Turner (1959b) that the rate of I^{131} release was not increased by reserpine which might have occurred had it antagonized the effect of thyroxine in controlling TSH secretion. They also confirmed that reserpine did not inhibit the effect of exogenous TSH. Further, Yamazaki and co-workers observed

that the return of TSH secretion following thyroxine was not clearly inhibited by reserpine thus questioning though not ruling out the conclusion of Moon and Turner (1959b) that reserpine suppresses the secretion of TSH.

The term "stress" is generally used to describe any experimental condition which causes an increase in adrenocortical activity as a result of a reflex discharge of ACTH from the pituitary. Such conditions have led to a decrease in thyroid activity in the rat and rabbit. The thyroid response to stress is thought to be due to a decrease in TSH secretion coincident with the increased secretion of ACTH and perhaps consequent upon it. Increased adrenal steroid secretion per se has no effect. Two types of stress have been suggested: neurotropic--the response to which is dependent on normal hypothalamic-pituitary connections, and systemic--which act on the transplanted pituitary or in the presence of median eminence lesions. The increase in adrenal activity and the decrease in thyroid activity which normally follow surgical trauma are not abolished in rabbits whose pituitary stalks are cut whereas the divergent responses of these two glands to emotional stress are abolished (Brown-Grant, 1961).

It has been known that thyroid activity is markedly reduced when the normal vascular relationship of the anterior-pituitary to the hypothalamus is blocked, though not to the level seen after hypophysectomy. A direct action of thyroxine upon anterior pituitary tissue resulting in a decrease of TSH secretion has been demonstrated. It is not necessary to invoke an action upon the hypothalamus in the intact animal to explain some results but such an action cannot be excluded. The response of the pituitary to a lowered level of circulating thyroid hormone is reduced but not abolished in animals where the normal vascular relationship to an intact hypothalamus is interrupted. The acute response of the thyroid to cold is dependent upon the presence of a pituitary in normal vascular relationship to an intact hypothalamus. Clearly, a simple feedback mechanism cannot account for the observed changes in thyroid activity.

Role of a hypothalamic neurohumor that would act upon the pituitary to stimulate TSH secretion has been proposed. The nature of such neurohumoral transmitter substance or substances for TSH as for any other trophic hormones is still unknown. Vasopressin may be concerned in the release of ACTH, while oxytocin has been suggested for the release of prolactin (Brown-Grant, 1961).

Unpublished reports of Brown-Grant (1961) indicate that high doses of commercial vasopressin (5 units/day) which cause ACTH release inhibit the release of I^{131}-labeled hormone from the thyroid gland of rabbits and guinea pigs.

III. Serotonin (5-hydroxytryptamine) and 5-hydroxytryptophan

A. General Pharmacology and Relationship with Reserpine

The occurrence of depressive symptoms in the course of treatment with reserpine stimulated research into the possible causes of the effective change and was soon coupled with observations on the liberation by reserpine of 5-hydroxytryptamine (5-HT) from its binding sites in platelets and the disappearance of 5-HT and catecholamines from the central nervous system in reserpinized animals.

The general pharmacological actions of serotonin have been reviewed exhaustively by Page (1954, 1958); Erspamer (1954b); and in a recent symposium edited by Lewis (1958). Extracts of rabbit gastric mucosa contained a pharmacologically active amine. It was called enteramine and chemically identified as 5-hydroxytryptamine. A hitherto known vasoconstrictor substance released from platelets on the coagulation of blood was isolated, characterized and named

serotonin (Rapport et al., 1948).

Serotonin has been found in the hypothalamus, midbrain, medial part of the thalamus and grey matter of the spinal cord (Amin et al., 1954). Paasonen et al. (1957), Bogdanski et al. (1957) and Kuntzman et al. (1961) noted the highest concentration of serotonin in brain stem, hypothalamus and limbic structures.

Endogenous production of serotonin starts with the amino acid tryptophan. Tryptophan is hydroxylated to form 5-hydroxytryptophan which in turn is decarboxylated to form 5-hydroxytryptamine (Undenfriend et al., 1953 a,b; Gaddum and Giarman, 1956). The major pathway of enzymatic destruction of serotonin in the mammal is by way of oxidative deamination by monoamine oxidase to form the urinary excretory products 5-hydroxyindole acetic acid (5HIAA) and others (Blaschko, 1952; Erspamer, 1954a; Sjoerdsma et al., 1957; McIsaac and Page, 1959; Titus and Udenfriend, 1954).

Support for a central role of serotonin in addition to other evidence comes from the fact that its effects are blocked by lysergic acid diethylamide (Shore, 1955) and the finding that central actions of reserpine are associated with the depletion of serotonin depots in the brain (Pletscher, 1956). Recognizing the important role of brain

stem, hypothalamus and limbic system in autonomic function and the localization of serotonin in these structures, Costa et al. (1962) ascribed a neurohumoral role to serotonin in brain. Endocrine disturbances caused by tranquilizers are presumed to result from their action on the diencephalon and particularly the hypothalamic region (Smelik and Sawyer, 1962).

Reserpine releases serotonin from intestine, platelets and brain. Serotonin administered to mice produced sedation and potentiated hexobarbital hypnosis by a central action, as did reserpine. Reserpine administered to dogs induced a marked increase in the urinary excretion of 5HIAA (Page, 1954, 1958). Out of a wide variety of other centrally acting drugs Rauwolfia alkaloids that cause sedation were found to liberate serotonin (Brodie et al., 1956; Carlsson et al., 1957).

On the basis of these works and their own Pletscher and his co-workers (1956) postulated that serotonin is important in normal brain function and that the central action of reserpine is mediated through its release into the body. Westermann (1962) has indicated that the central activity of reserpine is related to the blockade of serotonin storage.

Brodie and co-workers maintain that the release of

serotonin results from an inability of tissue to bind certain amines and is the basis of the sedative effects of reserpine. The importance of serotonin levels in the brain is not yet known (Killam, 1962).

As serotonin does not readily traverse the blood-brain barrier its effects could not be easily studied directly. Its precursor 5-HTP which passes into the brain and leads to raised cerebral serotonin levels (Udenfriend et al., 1957) has been reported to induce excitatory effects in animals. As the highest concentration of serotonin appeared to be in the hypothalamus and midbrain greymatter, Amin et al. (1954) and Brodie and Shore (1957) proposed a possible role for serotonin as a transmitter substance in the "trophotropic" division of the autonomic system. Gluckman and his co-workers (1957) showed the ability of serotonin to elicit central synaptic inhibition and the limitation of this effect to the brain. They suggested a "distortion of synaptic equilibrium" produced by serotonin or some other hypothetical "psychotogen" underlying the psychological disturbances.

Reserpine releases serotonin in its "free" form which is rapidly metabolized by monoamine oxidase. The effects of reserpine are delayed and parallel the depletion of serotonin rather than the level of drug in the blood (Brodie et al.,

1956). When the amines were pretreated with the monoamine oxidase inhibitor, iproniazid, the "free" serotonin accumulated and the sedative effects of reserpine were replaced by those of stimulation (Chessin et al., 1957).

The findings of Schanberg (1963) that 5-HTP and not serotonin is actively transported by the brain tissue and of Giarman and Schanberg (1958) that serotonin in the brain is associated with particulate fraction strongly support the concept that 5-HTP is actively transported in vivo into the brain cell, where it is decarboxylated to serotonin which is subsequently bound and stored or metabolized further. This differs from the proposed mechanism of serotonin storage in platelets where the high concentration of serotonin is maintained entirely by an active transport mechanism for this amine (Hughes and Brodie, 1959). Udenfriend and his coworkers (1957) have shown rapid penetration of 5-HTP into almost all body tissues, including the brain, and yielding serotonin in those organs containing 5-HTP-decarboxylase. Since serotonin itself when administered in equivalent quantities is rapidly metabolized and does not penetrate into the brain in measurable quantities it may be concluded that brain serotinin is normally synthesized in the central nervous system. 5-HTP converted to serotonin in the brain

maintains an increased brain serotonin concentration over a long period of time. However, it is suggested that small amounts of serotonin may enter the brain (McIssac and Page, 1959).

Studies with 5-HTP in animals have shown that an increase in brain serotonin can lead to marked central disturbance (Bogdanski, 1956) and effects resemble those seen after administration of reserpine and iproniazid (Shore, 1962). Brain serotonin levels after having been lowered by reserpine can be increased after administration of 5-HTP (Pletscher, 1956). This may be accompanied by the reversal of some of the pharmacological effects of reserpine. It was shown that brain levels ten times normal have been reached and maintained for several hours. The administration of 5-HTP to animals produces measurable increases of blood and tissue serotonin within fifteen minutes which reach a maximum in about one hour and persist for several hours (Udenfriend et al., 1957). Exogenous serotonin is rapidly metabolized by rats and rabbits and excreted, 50-80% being eliminated in the urine within 24 hours. Where excretion was 25% in 24 hours the animal developed paresis of the hind limbs, became comatose and died.

Intravenously administered serotonin is rapidly degraded to its inactive metabolite 5-HIAA. It must be considered that the effect of intracellular serotonin may be different from that of extracellular serotonin. Administration of 5-HTP results in an increase in intracellular as well as extracellular serotonin (Haverback, 1962). Intravenous administration of 5-HTP in doses of 25-50 mg over a 30 minute period in man had no effect on pulse, blood pressure, respiration or mental status though intestinal motility increased within 6-40 minutes and persisted for at least ninety minutes. It was conjectured that the enterochromaffin cells, where the largest store of serotonin is found, normally influence intestinal motility by releasing a humoral agent which acts on the neural elements within the intestinal wall as it was found that low concentration of serotonin administered to the outside of the intestine never increased peristalsis and that high concentration inside never abolished peristalsis (Bülbring and Lin, 1957).

Carlsson (1957) suggested that reserpine could act by "denaturing" the storage site so that it no longer localizes serotonin. The high temperature coefficient of reserpine action and the large number of serotonin molecules liberated by a single molecule of reserpine are facts compatible with

the concept that serotonin is kept within the cell by chemical forces. They also showed that although chlorpromazine and reserpine induce similar central effects the former does not liberate 5-HTP *in vivo* or *in vitro* indicating different mechanisms of these two drugs in altering central autonomic balance. That release does not occur by a procedure of displacement is supported by Hess *et al*. (1956). It was found that while the drug has only a brief sojourn (in hours) the levels of serotonin remain affected for several days.

B. Effect on Adrenal Activity

Moussatche and Periero (1957) found that serotonin caused a decrease in the adrenal gland ascorbic acid in intact but not hypophysectomized rats, indicating that serotonin may cause the release of ACTH from the pituitary.

Westermann (1962) has shown a close association between ACTH hypersecretion, decline in content of brain serotonin stores and the central activity produced by reserpine. They correlated the changes in adrenal activity to changes in serotonin stores rather than norepinephrine stores.

Rosenkrantz (1959) demonstrated a direct effect of serotonin on the adrenal cortex *in vitro*. Sodium retention occurred in human beings receiving serotonin (Blackmore,

1957). Erspamer (1954) reported chloride and water retention in rats, with serotonin.

As a result of the antioxidant property of serotonin, particularly in the presence of ascorbic acid, higher concentration of precursors of corticosteroid synthesis would be expected (Rosenkrantz, 1959). It was postulated that the release of aldosterone by serotonin could result in an abnormal electrolyte metabolism influencing kidney function (e.g., reabsorption of chloride and water) and brain activity (depolarization of membranes).

Rosenkrantz and Laferte (1960) further showed a profound influence of serotonin *in vitro*, with a high degree of specificity of chemical structure, on the adrenals. The activity of serotonin could be protected by iproniazid, showing that adrenal monoamine oxidase could be inhibited.

Thus evidence for both a direct and indirect action of serotonin on adrenals was available. Verdisca and coworkers (1961) confirmed the *in vitro* work of Rosenkrantz (1959, 1960) working on isolated intact adrenal glands of hypohphysectomized dogs. They showed a direct stimulatory action of serotonin on adrenals. However, 5-HTP and reserpine were found to be inactive. They also suggest that previously observed effects of reserpine on adrenal

steroidogenesis were not due to ACTH release but were the result of reserpine releasing serotonin from the brain. Connors and Rosenkrantz (1962) strongly suggest that the serotonin effect depends on a functional oxidative phosphorylation system.

C. Evidence for "Serotonergic" Fibers in the Central Nervous System

Heller et al. (1962), studying the effect of central nervous system lesions on brain serotonin, suggest that damage to one particular system of fibres is responsible for the effect on brain serotonin: the fibres of the medial forebrain bundle within the lateral hypothalamus. It is only the destruction of the areas contributing fibres to this region of the medial forebrain bundle that cause a decrease in serotonin (Harvey et al., 1963). It was found that the fall does not begin until sometime between 2-3 post operative days and is completed by the 12th day. Heller et al. (1962) and Harvey et al. (1963) indicate a possible neural origin for serotonin in the brain. Brodie and Shore (1957) postulated the existence of "serotonergic" fibres in the central nervous system. Such a hypothesis has been presently supported by Heller and Harvey. The latter did not find any correlation between the effects of a lesion on brain serotonin levels

and its effects on the body weight, brain weight, or irritability of the animal and suggest that the demonstration of a neural origin for serotonin does not provide any direct evidence for ascribing to this amine a function in either axonal or synaptic transmission.

D. Binding and Release in Relation to Reserpine Action

Green (1962) has reviewed the binding of biogenic amines in tissues. These amines have been found associated with granules which are distinct from mitochondria. They are held in loose linkage within the membranes. The granular fraction from brain containing serotonin also contains acetylcholine although it is regarded as unlikely that each substance is present in the same granule. Reserpine prevents the action of acetylcholine by way of sympathetic fibres by depleting the norepinephrine stores- the effective agent for sympathetic response. It is generally agreed that in sufficient doses reserpine depletes tissues of catecholamines, by causing their release. Reserpine, it is held, renders the brain incapable of binding serotonin which then flows free to exert the actions that have been assigned to reserpine. This hypothesis is not universally accepted but the fact that reserpine causes release of serotonin is irrefutable. It

produces a profound and persistent alteration of the cellular mechanism for binding amines as its continued presence is not necessary for activity. Possibly reserpine acts upon granules to control release and in addition acts upon cells to prevent uptake. An extragranular site is suggested by the observation that reserpine, though preventing uptake of serotonin by intact cells, did not prevent uptake by isolated brain particulates (Walaszek and Abood, 1959; Whittaker, 1961). The granules are not the only target for a compound to induce the release of amines. Differences among granules may explain some of the variations by organs in their response to drugs. Serotonin in brain and catecholamines in heart are more sensitive to reserpine than are the same amines in other tissues. A blockade of the binding sites should accelerate the rate of metabolism of an amine, because the amine, not being bound to tissue components is more accessible to enzymes which cause inactivation. Indeed, reserpine reduces the rate of uptake by tissues of epinephrine and norepinephrine, elevates the blood levels of these hormones and increases their rate of metabolism. A hypothesis has been suggested that reserpine could effect the uptake of exogenous amines by preventing granules from binding amines and/or decreasing the number of granules.

Shore (1962) reviews evidence that there appear to be two mechanisms of serotonin uptake by platelets. The primary action of reserpine on amine stores is to prevent the uptake of small amounts of amines. Introduction of reserpine then leads to depletion of the platelets as the amine within the cell passes out of the cell by passive diffusion and cannot be pumped back again. Reserpine-insensitive uptake predominates at high serotonin concentration. A similar mechanism for amine concentration in brain was looked for using 5-HTP in normal and reserpinized rabbits. It was seen that a serotonin concentrating mechanism exists in brain as well as in platelets. However, the findings of Schanberg (1963) that 5-HTP and not serotonin is actively transferred by brain tissue differs from the above findings suggesting different mechanisms in brain and platelets for concentrating serotonin. Platelets maintain a high concentration of serotonin entirely by an active transport mechanism for this amine (Hughes and Brodie, 1959).

It was found that the uptake of small amounts of catechol amines by brain slices occur by a reserpine sensitive mechanism similar to that reported for serotonin and catecholamines in platelets. These results are consistent with the view that a continual mechanism operates to pump the amine

across the membrane into the cell and that the concentration of norepinephrine taken up reaches a plateau only when levels of the amine within the cell are sufficiently high that passive outward diffusion just balances the active inward pumping action.

All experimental results in this direction are consistent with the hypothesis that reserpine lowers the content of both serotonin and catecholamines in tissues primarily by blocking a transport mechanism which pumps the amines across a membrane into the cell. Depletion of a tissue amine content then is pictured as a consequence of the uncompensated passive leaking of the amine from its intracellular pool or pools.

IV. Monoamine Oxidase (MAO) and Monoamine Oxidase Inhibitors

Spector et al. (1960) have recently presented and reviewed evidence which strongly suggests that monoamine oxidase is responsible for the metabolism of catecholamines and serotonin in tissues where it may regulate the level of stored amines.

The enzyme is essentially intracellular and its substates are catecholamines and some of their precursors, tryptamine and serotonin (Blaschko, 1952). Monoamine oxidase has been

shown to occur in mitochondria (Hawkins, 1952). Sjoerdsma (1955) concluded that MAO is the major pathway of serotonin metabolism *in vivo*. The only catalyst of the biological inactivation of serotonin hitherto studied is monoamine oxidase (Blaschko, 1957).

It was suggested that the stimulating effects of iproniazid (1-isopropyl-2-isonicotinyl hydrazide) may be due to the inhibition of MAO which had been investigated by Zeller and his co-workers (1952b). Udenfriend (1957b) reported a rise in brain serotonin in animals after administration of iproniazid. 5-HIAA occurs in normal urine and the amount increases following the administration of serotonin in rat and dog (Titus and Udenfriend, 1954).

Zeller (1952a) showed a marked *in vitro* and *in vivo* inhibition of mammalian MAO by ipronazid, suggesting that iproniazid not only enters the cells but also penetrates the mitochondria. They observed that the administration of iproniazid to patients and animals can lead to accumulation of sympathomimetic amines with ensuing effects on the autonomic nervous system. The restoration of ACTH after single injection of iproniazid took about five days in rats. The reappearance of MAO might not have been due to a reactivation of the original enzyme molecule but to the formation of new MAO (Aprison,

1961). The loss of serotonin and noradrenaline from brain tissue, caused by reserpine, is greatly delayed by pre-treating the animals with MAO inhibitors (Shore et al., 1957; Pletscher, 1956) and then reserpine causes excitation instead of sedation (Brodie et al., 1956; Chessin et al., 1957). While it has been suggested that MAO inhibitors may prevent amine release by reserpine, this has not been substantiated (Green, 1962). It seems that a more likely explanation is that the action of the inhibitors is due to prevention of destruction of the serotonin (Spector et al., 1960a).

The pressor effect of reserpine in the rat and dog is inhibited by adrenaline antagonists showing release of catecholamines by reserpine. Since the effects of injected adrenaline and noradrenaline are but slightly increased by MAO, and as MAO is an intracellular enzyme, it seems that more of the pressor amine released by reserpine may escape from the cells when the enzyme is inhibited (Green, 1962). Clinical improvement of various degrees has been claimed to follow the administration of iproniazid and other inhibitors in the treatment of depressive states. This action seems to be linked in some way with the ability of iproniazid to prevent the enzymatic destruction of amines in brain tissue and at present serotonin seems to be one of the most important of these amines (Green,

1962). Sjoerdsma et al. (1955) observed that the effects of serotonin in vivo can be prolonged following treatment with iproniazid. They speculated that the only physiologic action of MAO is on serotonin and that MAO is not related to adrenergic function (Griesemer et al., 1953).

The prolonged effects of reserpine are thought to be associated with persistent impairment of the capacity of the body to maintain serotonin in the bound form which normally protects the amine from the action of the active enzyme, MAO. Reserpine administration results in a persistent flow of serotonin from cells which have lost part of their capacity for storing the serotonin, as reserpine does not effect the enzyme responsibile for synthesis of serotonin. It has been further speculated that free amine might stimulate the central parasympathetic centres producing signs of typical reserpine action. Usually parasympathomimetic effects of reserpine have been associated with a low concentration of free serotonin in brain. With a high concentration sympathetic predominance occurs resembling the behavior of acetylcholine, which in high concentration blocks its own actions at peripheral ganglia. A neurohumoral role for serotonin in the parasympathetic division of the central autonomic system has been suggested.

Blockage of central parasympathetic centres could unmask the antagonistic sympathetic centres and result in sympathetic predominance (Shore et al., 1957). They administered iproniazid two hours before reserpine and observed excitation and sympathomimetic effects similar to those observed after administration of lysergic acid diethylamide (LSD) or high doses of 5-HTP. They associated the effects with high levels of serotonin in brain. The effects of 5-HTP have been shown to be pronounced when destruction of serotonin has been blocked by iproniazid. Serotonin and iproniazid interrupt pregnancy in mice probably due to interference with the normal humoral mechanism necessary for maintenance for pregnancy; the inhibition of the pituitary has been cited. However, the possibility of a direct toxic effect on uterine contents exists (Botros et al., 1962).

Iproniazid is reported to cause selective blockade of sympathetic ganglia. Whether the impairment of sympathetic functions with amine oxidase inhibitor results from an imbalance of amine concentration either in the brain or effector cell is uncertain (Green, 1962).

Certain behavioral changes in pigeons have been attributed to central action of raised level of serotonin following iproniazed (Aprison, 1961). Monoamine oxidase inhibitors

differ considerably from one another in their interaction with reserpine. Spector et al. (1961) indicated that MAO inhibitors do not block the reserpine induced release of amines from their binding sites and their principal action in the presence of reserpine is to prevent the destruction of the amines by MAO. However, Schanberg and Giarman (1962) feel that iproniazid interacts with reserpine at the binding sites of serotonin. Brain levels of both serotonin and norepinephrine can be maintained above control values by the simultaneous administration of MAO inhibitor, nialamide, to monkeys. They suggested that nialamide may exert more than the single action of inhibition of MAO.

Iproniazid blocks the reserpine effects for an extremely long duration as shown by amine oxidase inhibition of rat brain in vivo and the blocking of reserpine ptosis in rats for about 20 days. Iproniazid is not able to reverse the reserpine effects but blocks them if given prior to reserpine thus acting as an indirect stimulant as compared to amphetamine a direct stimulant, which causes reversal of reserpine responses. Iproniazid, however, potentiates the excitatory activity of 5-HTP (Randall, 1959).

All sections of the guinea pig brain show the same $C-^{14}$ concentration-time relationship, with no one area having a consistently higher content than the others, when $C-^{14}$ labeled reserphine is administered (Sheppard et al., 1957).

If the enzymic inhibition by iproniazid is to be accepted it seems that MAO inhibition is the most important reaction. However, iproniazid and its analogues inactivate many enzymes, including the one involved in metabolism of 5-HTP: decarboxylase (Zbinden et al., 1960). They observe that although serotonin, epinephrine and norepinephrine are the frequently considered amines effected by MAO inhibitors there are other known and unknown amines which might be important.

Further the adrenergic response to reserpine in iproniazid-pretreated rats have been largely attributable to the release of pressor catecholamines (Franco-Browder, 1958). Hyperactivity of animals has been correlated more to the raised levels of norepinephrine than to serotonin (Spector et al., 1959). These authors, however, note that in the face of diversified pharmacological effects of these drugs it is difficult to assign the complete role to norepinephrine and serotonin. An unknown amine may be responsible because some of the MAO inhibitors, including iproniazid, elevate brain serotonin levels and not norepinephrine levels without

causing any excitement. They have suggested an intracellular regulatory role of MAO in regulating serotonin and norepinephrine levels. The amines can only exert their effect after the storage sites have become saturated and amines are spilled to receptor sites, thus explaining the delayed effect of MAO inhibitors.

V. 2-Bromo-D-Lysergic Acid Diethylamide (BOL-148) a Specific Serotonin Antagonist

Since the central effects of reserpine have been attributed to serotonin release the antagonistic action of lysergic acid diethylamide (LSD) has been explained by its antiserotonin effect. However, the chief cause of central LSD effects has been proposed to be central sympathetic stimulation, not related to the antiserotonin effect of LSD (Taeschler, 1956).

BOL, a lysergic acid derivative, has a specific antiserotonin effect. It cannot be argued that BOL lacks cerebral actions because it does not penetrate into the brain tissue; the sedative action it produces is a central effect. Further BOL could be detected in extracts of brain (Cerletti and Rothlin, 1955).

Specific antagonism by BOL on serotonin action on rat and guinea pig uterus as well as on guinea pig ileum and rabbit intestines has been shown (Sollero et al., 1956). BOL lacks

the sympathetic stimulatory effect of LSD; it does not antagonize the sedative- and barbiturate-potentiating action of reserpine although it blocks the serotonin-induced potentiation of barbiturates to the same extent as LSD. It, therefore, appears that reserpine and injected serotonin in mice do not have a common mechanism in potentiating the barbiturate hypnosis (Taeschler and Cerletti, 1957). It was suggested by Salmoiraghi and Page (1957) that reserpine-induced potentiation of hexobarbital hypnosis is not directly mediated by free serotonin released from body depots (Pletscher et al., 1955, 1956).

MATERIALS AND METHODS

I. Experimental Animals

One hundred and forty-four female rats of the Carworth CFN strain were used in a series of two experiments.

In Experiment I the rats weighed 180-238 gms and were approximately three months old. In Experiment II the rats weighed 237-231 gms and were approximately 5 months old.

All rats were ovariectomized by the dorsal approach under ether anesthesia four to six weeks before the start of the treatments, and were apportioned to nine groups of six rats each in Experiment I and to fifteen groups of six rats each in Experiment II.

The rat room was kept at a temperature of $76^{\circ} \pm 1^{\circ}$ F. uniform humidity and lighting hours were maintained throughout the experiment except for one day when the temperature went up to 81° F. Its implications are mentioned in the results. The animals were fed a diet consisting of:

35 parts corn	15 parts skim milk powder
25 parts wheat	3 parts yeast
6 parts alfalfa meal	10 parts linseed meal
5 parts corn meal	1 part iodized table salt

Tap water and feed were provided ad libitum. All rats were handled for about a week before any experimentation.

II. Treatment and Dosage

Reserpine: Reserpine solution was prepared by dissolving 15 mg of the powder (Merck and Co., Inc.) in 0.1 ml of glacial acetic acid and adding 0.2 ml of propylene glycol, 0.2 ml of ethanol and 74.5 ml distilled water.

200 μg/Kg was administered subcutaneously daily either when given alone or in combination with other drugs, unless mentioned otherwise.

Serotonin (5-hydroxytryptamine , Nutritional Biochemical Corporation): 12 mg/Kg of Serotonin, as creatinine sulfate was dissolved in distilled water and injected daily both when given alone or in combination with other drugs.

Iproniazid phosphate (1-isonicotinoyl-2-isopropyl hydrazine phosphate) was administered intraperitoneally in distilled water. 100 mg/Kg was injected as the initial dose followed by 50 mg/kg every fourth day. The same time and dose sequence was maintained when used in combination with other drugs. Iproniazid was injected two hours before when given in combination with reserpine, Serotonin or 5-HTP.

2-bromo-D-lysergic acid diethylamide bitartrate (BOL-148, Sandoz Pharmaceuticals): BOL-148 was dissolved in distilled water and injected intraperitoneally, 500 μg/Kg daily, both when given alone or in combination. When given

with reserpine, 5-hydroxytryptophan (5-HTP), and Serotonin BOL-148 was administered half an hour before the other drugs.

DL-5-hydroxytryptophan (5-HTP, Nutritional Biochemicals Corporation): 5-HTP, the precursor of Serotonin, was dissolved by mixing with a few drops of 4N hydrochloric acid and adding distilled water. Fresh solution was made each day. 50 mg/Kg of 5-HTP was injected subcutaneously in daily doses.

III. Procedure

The present study was divided into two series of experiments.

Experiment I included nine groups of six rats each. These were subjected to a long term treatment of forty days. The groups were divided according to the treatments given, as follows:

Group Number	Treatment	Frequency and Sequence
I	Reserpine	Daily
II	Serotonin	Daily
III	Iproniazid	Every 4th day
IV	BOL	Daily
V	Iproniazid + Reserpine	Iproniazid (100 mg/Kg) was injected on the first day followed 2 hours later by reserpine. Henceforth Iproniazid (50 mg/Kg) was injected every 4th day and reserpine daily. Reserpine always followed Iproniazid.

VI	Iproniazid + BOL + reserpine	Same sequence as in Group V was used with the addition of daily injection of BOL one-half hour before R injection.
VII	Iproniazid + Serotonin	Same sequence and frequency as in Group V.
VIII	Iproniazid + BOL + Serotonin	Same sequence as in Group VII with the addition of daily injection of BOL one-half hour before Serotonin.
IX	Saline	Daily subcutaneous injection.

Experiment II included fifteen groups of six rats each. These were subjected to a short term treatment of fourteen days as follows:

Group I to VIII were given the same frequency and sequence of treatment as in Experiment I. In addition the following groups were included:

Group Number	Treatment	Frequency and Sequence
I'	5-HTP	Daily
II'	BOL + 5-HTP	Daily injection with BOL half an hour before 5-HTP.
III'	Iproniazid + 5-HTP	First day Iproniazid (100 mg/Kg) was injected followed by 50 mg/Kg every fourth day. 5-HTP daily and two hours after Iproniazid.
IV'	Iproniazid + BOL + 5-HTP	Same as in Group III with the addition of daily injection of BOL half an hour before 5-HTP.
V'	BOL + Reserpine	Daily with BOL half hour before reserpine.
VI'	Iproniazid + BOL	BOL injected daily one-half hour after Iproniazid. Frequency and sequence of Iproniazid as in other cases.
VII'	Saline	Daily subcutaneous injection.

Various measurements as detailed in the next section were made during the course of the experiment. Rats were killed on the 40th and 14th days of treatment, respectively. Thyroid, adrenal and thymus weights were recorded on a Roller-Smith torsion balance and preserved for further examination.

IV. Measurements

A. Energy Metabolism

The energy metabolism of each rat was determined every fourth day using oxygen consumption by a closed circuit method (MacLagan, N. F. and M. M. Sheahan, 1950), as modified by E. P. Reineke.

A unit of twelve desiccators was used (Fig. 1). Each desiccator, 6 1/4" in diameter, of known volume and containing soda lime for absorption of carbon dioxide was connected to a mercury manometer. The animals were allowed to rest for about fifteen minutes and the desiccator charged with oxygen of constant amount. A further one-half hour was allowed for the animals to relax. The oxygen consumption was determined for two one-half hour periods from the pressure difference in the mercury manometers.

Rats were kept off feed for eighteen hours before each determination. The measurements were made at approximately

the same time of day. When drug injections were scheduled for a given day, all determinations were made prior to injection.

Three to four control measurements were made for each rat before the start of the treatment. Percent deviations from the average control determination were recorded.

B. I-131 Turnover Rates

Chronic treatment: On the 34th day of treatment each rat was injected with 10 microcuries of carrier-free I-131 as NaI. In vivo external thyroid counts were taken daily under ether anesthesia for six days. A heavily shielded scintillation detector with a 1" NaI crystal connected to a count-rate meter (Nuclear, Model 1620 and Esterline-Angus recorder) was used. The counter was mounted beneath an aperture of 1.0" diameter and located 4" from one end of a 1" x 16" x 14" lead plate. Body background was measured over the gastric region.

Thyroidal I-131 uptake as percent injected dose was computed as follows:

$$\text{Thyroid uptake} = \frac{\text{Thyroid count} - \text{1/2 body background} - \text{1/2 general background}}{(\text{Standard count} - \text{general background}) \times 10} \times 100$$

A standard solution one-tenth of the amount of injected dose was made.

Acute treatment: On the 10th day of the treatment three microcuries of carrier-free I-131 as NaI was injected into each rat intraperitoneally. Thyroid counts were taken daily for 4-5 days.

The counting procedure was the same as described earlier except that a scintillation counter with a two-inch NaI crystal was used together with a pulse height analyzer (Nuclear, Chicago, Model 1810, and Esterline-Angus recorder).

C. Total Iodine Content of the Thyroid

The total iodine in the thyroid was determined by an alkaline fusion-ceric sulfate method modified from a procedure (Barker et al., 1951) for the PBI of blood plasma. The reaction used was based on the work of Sandell and Kolthoff (1939) who studied the reaction of iodine as a catalyst in the following reaction:

$$2\ Ce^{++++} + H_2ASO_3^- + H_2O = 2\ Ce^{+++} + H_2ASO_4^- + 2H^+$$

The two lobes of thyroid from each rat were cleaned of extraneous tissue and weighed immediately on a Roller-Smith torsion balance. They were then placed in individual vials containing Dietrich fixative. Before alkaline fusion the fixed thyroid was weighed again and divided into two. One lobe was used for histology. The other lobe was weighed,

transferred to a nickel crucible containing 0.1 ml of 4N sodium carbonate solution (212 gm/liter) and placed in an oven at 90-95° C to dry overnight.

The dried samples were ashed for about two hours at 600° C ± 10° in an electric muffle furnace equipped with a pyrometer ranging up to 1100° C and then allowed to cool. The ash was dissolved in 2 ml of 2N hydrochloric acid (200 cc conc. HCl/liter) followed by 2 ml of 7N sulfuric acid (196 cc conc. H_2SO_4/liter). Total volume was made to 25 ml by adding 21 ml distilled water. Contents were mixed thoroughly and transferred to a pyrex glass centrifuge tube. The insoluble carbonacious material was allowed to settle and if necessary removed by centrifuging.

Two 0.5 ml aliquots, for duplicate analysis, were pipetted from 25 ml total volume into round bottomed optically calibrated colorimeter tubes. 0.5 ml of arsenious acid was added (using a "Blow-out" pipette) and total volume made to 5.5 ml by adding distilled water. Final volume after later addition of 0.5 ml of ceric ammonium sulfate was 6.0 ml. The tubes were mixed well and then placed in a water bath, accurately regulated to 27° C, for a 15 minute equilibrium period.

0.5 ml of ceric ammonium sulfate was added successively to each tube at 60-second intervals using a "blow-out" pipette and the tubes were thoroughly mixed and returned to the water bath.

Exactly 15 minutes after addition of ceric ammonium sulfate the percent transmittance was read in a Coleman Universal spectrophotometer Model 11 using a PC-4 blue filter and a wave length of 430 millimicrons.

The initial setting of the instrument (100% T.) was made against distilled water. A reagent blank was prepared exactly as described above except that the thyroid sample was omitted.

The reading of the reagent blank was subtracted from the reading of the iodine standard or reading of unknown samples. The concentration of iodine was read directly from a standard curve plotted on arithmetic graph paper. The values were converted to μgm I/mgm of thyroid tissue and to total iodine in the whole thyroid. Necessary connections for dilutions were made.

All determinations were performed in duplicate. A control standard of known concentration of iodine (Hycel's standard iodide reagent) and a reagent blank without thyroid tissue were included with each series of unknown samples.

All reagents used were of the highest purity obtainable. All solutions were made in water redistilled once in an all-glass still. Maximum care was taken in cleaning and drying glassware. Temperature and time factors were accurately controlled.

D. Body Weights

Changes in body weights were recorded. Body weights, in gms, were recorded every fourth day, starting with the first day of treatment. Final body weight was recorded on the last day of the experiment after sacrificing the animals. The percent deviation from the initial body weight was calculated.

E. Organ Weights

The rats were killed on the 40th and 14th days of treatment in Experiments I and II, respectively. Thyroid, adrenal and thymus weights were recorded using a Roller-Smith torsion balance and were placed in individual vials containing Dietrich's fixative for further examinations.

F. Computations

Standard errors and tests of significance of differences (Students "t" test) were computed by standard statistical methods. Regression equations for the thyroidal I^{131}

turnover data were calculated by the method of least squares by use of the MSU Fortran computer. The program employed was devised by Dr. E. S. Koushanpour.

The thyroid secretion rate was estimated making the following series of calculations: The notations employed are those of Brownell (1951).

$$K'_4 = b \text{ (semi-log slope)} \times 2.302 \qquad (1)$$

$$K_4 = K'_4 \times \frac{1}{1-\mu} \qquad (2)$$

where μ = antilog of 'a' intercept or zero hour uptake.

$$\text{Estimated daily thyroid secretion} = K_4 \times \text{total thyroid iodine } (\mu g) \times 1.54 \times 1.55 \qquad (3)$$

Preliminary comparisons (Reineke, unpublished) between values for TSR of rats computed by equation (3) and the thyroxine substitution method of Reineke and Singh (1955) show satisfactory agreement. Inasmuch as the procedure has not been published, however, some explanation is required. It is assumed (1) that the iodinated compounds in the thyroid are uniformly labeled with I^{131}, (2) that all the iodine released from the thyroid is in the form of thyroxine (T_4) or triiodothyronine (T_3) and (3) that these two compounds are released in the same proportions in which they occur in the

thyroid. Then, output rate (K_4) x thyroidal iodine content = hormonal iodine released. This value x 1.54 = the thyroxine equivalent. However, this fraction includes some T_3 which is 4.85 x as potent per unit of iodine as T_4 in suppressing TSH release in the rat (Reineke, unpublished). The rat thyroid contains 6 parts T_4 to 1 part T_3 iodine (Pitt-Rivers and Rall, 1961). Hence, the potency of the compounds released can be assessed as follows:

	T_4	T_3	Totals
Parts	6.00	1.00	7.00
Potency	1.00	4.85	
Activity	6.00	4.85	10.85

To adjust the T_4 equivalent in proportion to the potency of the mixture of T_4 and T_3 assumed to be released we use the factor: $\frac{10.85}{7} = 1.55$.

Calculations for energy metabolism were made as follows:

$$O_2 \text{ consumption in ml/hr (STP)} = (V-Va) \times \frac{P}{760} \times \frac{273}{273+t} \times \frac{60}{T}$$

where V = net volume of unit in ml
Va = volume of animal (assuming 1 gm = 1 ml)
P = pressure difference in mm Kg
t = temperature in the desiccator
T = time of determination in minutes

Calories heat produced/24 hrs = O_2 consumption x 4.825.

$$\text{Energy metabolism} = \frac{\text{Cal/24 hrs}}{\text{KgW}\cdot^{.75}} .$$

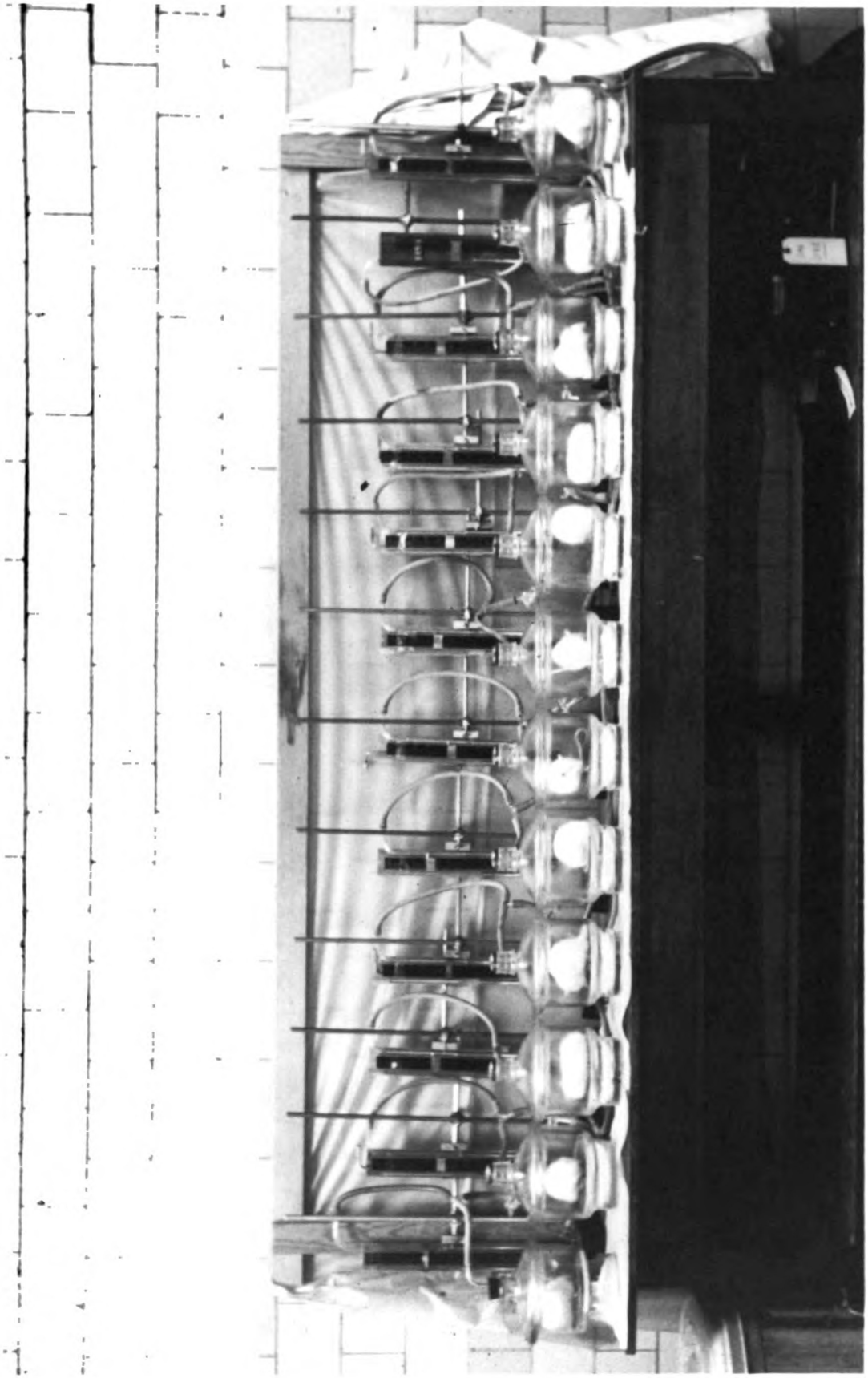


Figure 1: The apparatus used for the determination of energy metabolism.

RESULTS

It has been observed that a single injection of iproniazid inhibits monoamine oxidase for a prolonged period (Randall, 1959). Therefore, in these experiments iproniazid was injected every 4th day in order to avoid its toxic effects which normally ensue following daily treatment and to maintain a high level of serotonin. Iproniazid when given simultaneously with reserpine or following reserpine is not able to reverse the reserpine effects. On the other hand pretreatment with iproniazid blocks the reserpine action probably through the inhibition of monoamine oxidase and hence maintaining high levels of serotonin in the central nervous system. In this study, therefore, iproniazid was injected two hours prior to the administration of reserpine, serotonin or 5-hydroxytrophan (5-HTP).

Administration of reserpine, serotonin or 5-HTP to rats pretreated with iproniazid has been shown by many workers to cause (a) marked increase in peripheral and central levels of serotonin and (b) central excitation. Reserpine effects have been attributed to the release of serotonin by this drug (Pletscher et al., 1956).

BOL has been shown to be a specific serotonin antagonist.

It was of interest to see the effect of BOL on reserpine and serotonin actions. It was injected daily one-half hour before injections of reserpine, serotonin or 5-HTP so that it could exert its effects before serotonin started being metabolized by monoamine oxidase.

5-HTP but not serotonin has been reported to enter brain tissue. Therefore, to distinguish between the peripheral and central effects of serotonin, 5-HTP was used in some groups of rats alone and in conjunction with iproniazid and BOL:

I. General Effects of the Drugs

The effects of reserpine were noticeable after four days of treatment in these experiments. Thereafter, rats showed sedation and gradually a very much reduced feed and water intake. In chronically reserpinized rats 50% mortality was observed on the 17th-18th days of treatment. Therefore, after the 18th day of treatment reserpine was administered on alternate days in the same dose only to the group of rats receiving reserpine alone. The groups of rats receiving reserpine in conjunction with other drugs continued to get reserpine daily. After the 20th day there was less sedation and a gradual increase in feed and water intake was

noticed. Diarrhea was not observed at any stage of these experiments.

Iproniazid antagonized the action of reserpine on feed and water intake and sedation. BOL did not reverse these effects of iproniazid. No signs of excitement were noticed in the group of rats receiving reserpine that were pretreated with iproniazid. On the other hand these rats showed a mild degree of sedation on the 3rd and 4th days of iproniazid injection. BOL did not show any signs of antagonism to this iproniazid action. No mortality was encountered in acute treatment of 16 days. However, the effects on feed and water intake and sedation were the same. The group of rats receiving 5-HTP after pretreatment with iproniazid died within 6 hours of 5-HTP injection. The administration of BOL one-half hour before 5-HTP injections in groups of rats pretreated with iproniazid prevented this mortality.

Rats receiving daily subcutaneous injections of serotonin showed a tendency to lose hair and thickening of skin at the site of injections within 10 days. This became very marked after chronic treatment of 30 days. This is in agreement with the results of MacDonald et al. (1958). BOL, in the doses given, effectively antagonized these effects of serotonin in both loss of hair and thickening of skin.

The groups of rats undergoing chronic treatment with serotonin alone or a combination of iproniazid and serotonin, neither showed any signs of sedation nor any extrovert manifestation of peripheral or central pharmacological effects. However, the group of rats subjected to acute treatment with serotonin were sedated within half an hour of its injection. Signs of paresis of the hind limbs were noticeable. The animals were unresponsive to external stimuli like sound and pinching of toes. Generalized numbness was present. All of these effects were much pronounced if serotonin injections followed two hours after the injection of iproniazid. BOL very effectively antagonized the potentiation of serotonin effects by iproniazid. These results indicate rapid metabolism of serotonin. These results indicate that iproniazid is able to inhibit peripheral MAO. This is contrary to the belief of Udenfriend et al. (1957) that injected iproniazid has little effect on peripheral MAO. They maintained that it is only the central MAO which is inhibited by iproniazid. These results indicate the effectiveness of BOL, iproniazid and other drugs in the dosage used in this study.

II. Effects on Adrenal, Thymus and Thyroid Weights

The influence of various treatments on adrenal and thymus weights is presented in Tables 1-4 and on thyroid weights in Table 7. The chronic treatment (40 days) with reserpine produced no significant change in adrenal weights when administered alone or in combination with iproniazid on the one hand and iproniazid and BOL on the other. Acute treatment with reserpine resulted in significant hypertrophy of adrenals. Concomitant administration of BOL and reserpine did not block this hypertrophy. However, iproniazid alone or in combination with BOL blocked the reserpine effect on adrenals to a significant degree. Thus BOL did not antagonize the "normalization" of adrenals as a result of concomitant administration of iproniazid and reserpine. Iproniazid and BOL when given alone or in combination had no effect on adrenal weights.

No definite conclusions could be derived from the results on thymus weights of rats subjected to various treatments, though the thymuses showed a tendency toward a decrease in weight with increase in adrenal weight. However, the thymuses of chronically reserpinized rats were not significantly different from controls whereas the thymus in acutely

Table 1: The responses and changes in adrenal and thymus weights after chronic treatment with various drugs.

Group no.	Treatment	No. of animals	Body wt (gms)	Adrenal wt; mg/100 gm body wt	Thymus wt; mg/100 gm body wt
I	Reserpine (R)	3**	220	20.30 ± 0.62*	88.51 ±14.66*
II	Serotonin (5-HT)	6	223	22.78 ± 2.20	93.20 ±10.77
III	Iproniazid (IP)	6	235	17.87 ± 1.07	127.68 ± 9.70
IV	BOL	4	226	15.97 ± 0.59	103.11 ± 8.38
V	IP+R	5	219	14.64 ± 1.08	99.68 ± 8.98
VI	IP+BOL+R	6	220	15.59 ± 1.12	84.99 ± 7.73
VII	IP+5-HT	6	215	23.49 ± 0.86	74.89 ± 7.48
VIII	IP+BOL+5-HT	6	217	17.85 ± 1.38	88.79 ± 9.73
IX	Control	6	239	18.81 ± 1.49	115.46 ±10.86

*Standard error of the mean
**Three rats died between 17th and 18th day

Table 2: 't' and P values of adrenal and thymus weights as compared with the controls, after a chronic treatment with various drugs.

Group no.	Treatment	No. of animals	t		P	
			Adrenal	Thymus	Adrenal	Thymus
I	Reserpine (R)	3	0.6703	1.4021	NS	NS
II	Serotonin (5-HT)	6	1.4951	1.4702	NS	NS
III	Iproniazid (IP)	6	0.4913	0.8393	NS	NS
IV	BOL	4	1.4765	0.9007	NS	NS
V	IP+R	5	1.8412	1.1214	NS	NS
VI	IP+BOL+R	6	1.7235	2.2838	NS	0.10
VII	IP+5-HT	6	2.7155	3.0756	0.05	0.05
VIII	IP+BOL+5-HT	6	0.4723	1.8317	NS	NS
IX	Control	6				
	Reserpine IP+R	6 6	3.7565			
	Reserpine IP+BOL+R	6	2.9323			
	Reserpine IP+5-HT	6 6	2.4046			

reserpinized rats could not be discerned for proper removal. The thymuses from the group of rats receiving reserpine and BOL were significantly different from controls. The small thymuses in the second series of experiments was probably due to aging of the rats.

The thyroids did not show a significant change in weights with any of the treatments.

Serotonin alone had no effect on adrenal weights under either chronic or acute treatments. Iproniazid is known to inhibit monoamine oxidase within 2 hours of injection, delaying the metabolism of serotonin. This inhibition of monoamine oxidase has been shown to persist for a long period after a single injection of iproniazid reaching a peak of 90% inhibition at 18 hours and thereafter about 70% inhibition is maintained up to 5 days (Randall, 1959). In the present study a high level of monoamine oxidase inhibition was maintained by injecting iproniazid every 4th day before serotonin injection. Serotonin was injected daily. This treatment, acute or chronic, resulted in adrenal hypertrophy and a reduction in thymus weights. BOL effectively blocked this hypertrophy of adrenals under both treatments.

Brain tissue has been shown to concentrate serotonin from 5-HTP. 5-HTP when given alone had no effect on adrenal

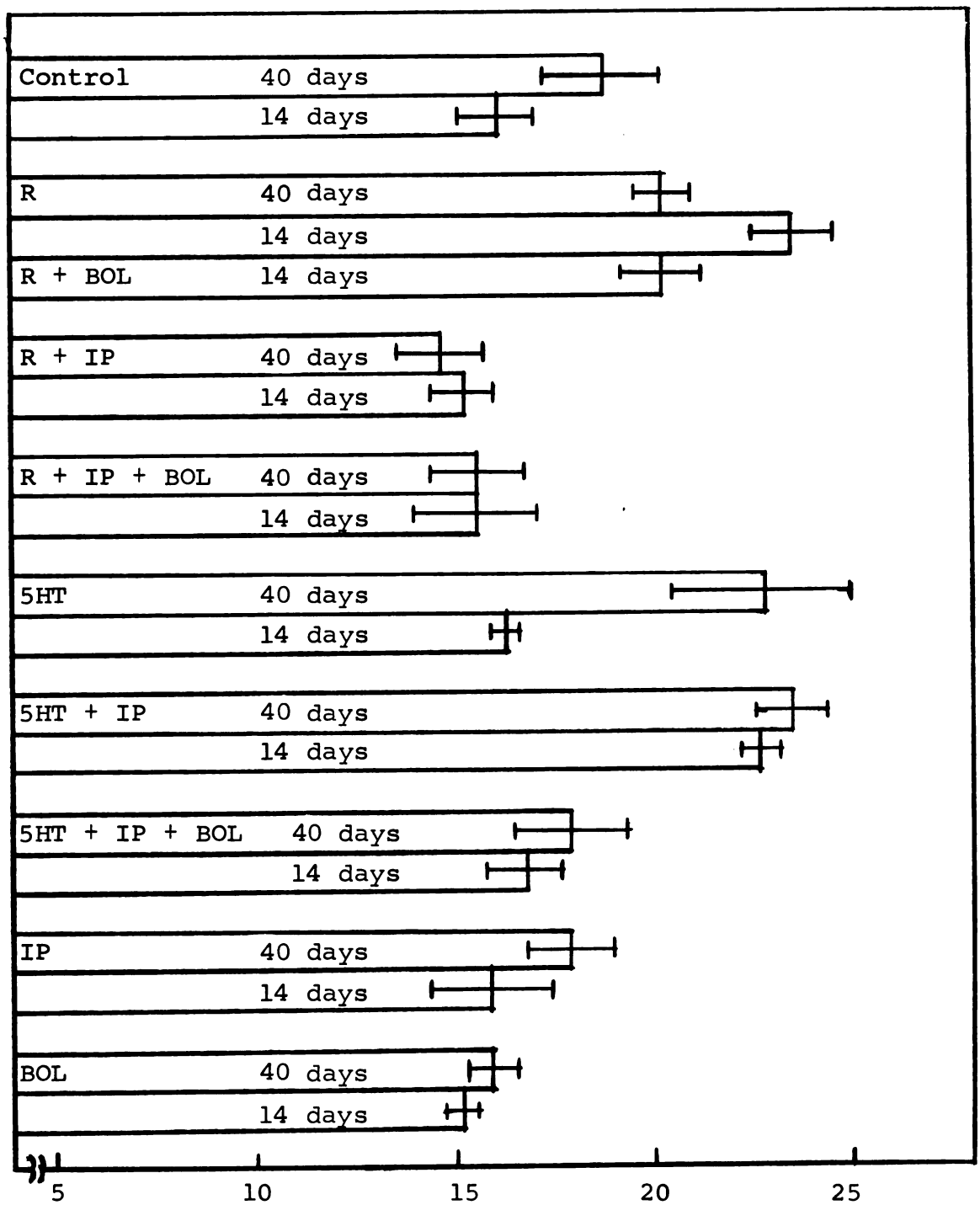


Figure 2: The responses and changes in adrenal weights after acute treatment and chronic treatment with various drugs.

Table 3: The responses and changes in adrenal and thymus weights after acute treatment with various drugs.

Group no.	Treatment	No. of animals	Body wt (gms)	Adrenal wt; mg/100 gm body wt	Thymus wt; mg/100 gm body wt
I	Reserpine (R)	6	217	23.46 ± 0.97*	-***
II	Serotonin (5-HT)	6	258	16.30 ± 0.32	19.99 2.69*
III	Iproniazid (IP)	6	243	15.94 ± 1.48	25.89 ± 5.29
IV	BOL	6	264	15.22 ± 0.43	30.96 ± 4.45
V	IP+R	4	267	15.30 ± 0.74	16.25 ± 2.57
VI	IP+BOL+R	6	258	15.63 ± 1.45	22.00 ± 3.58
VII	IP+5-HT	5	222	22.70 ± 0.47	14.87 ± 4.22
VIII	IP+BOL+5-HT	5	235	16.82 ± 0.90	13.55 ± 2.25
I'	5-HTP	6	272	15.74 ± 0.93	39.85 ± 3.52
II'	BOL+5-HTP	6	289	14.10 ± 1.24	28.21 ± 1.25
III'	IP+5-HTP**	6	-	-	-
IV'	IP+BOL+5-HTP	6	234	20.58 ± 0.83	23.55 ± 3.92
V'	BOL+R	6	234	20.33 ± 1.00	15.79 ± 2.07
VI'	IP+BOL	6	246	17.11 ± 0.91	36.59 ± 2.77
VII'	Control	6	272	16.15 ± 0.93	48.76 ± 9.59

*Standard error of mean
**The rats died six hours after the injection of 5-hydroxytryptophan (5-HTP)
***The thymuses had atrophied to an extent that they could not be dissected out.

Table 4: 't' and P values of adrenal and thymus weights, as compared with the controls, after an acute treatment with various drugs.

Group no.	Treatment	No. of animals	t		P	
			Adrenal	Thymus	Adrenal	Thymus
I	Reserpine (R)	6	5.4188		0.01	
II	Serotonin (5-HT)	6	0.1517		NS	
III	Iproniazid (IP)	6	0.1199		NS	
IV	BOL	6	0.9042		NS	
V	IP + R	6	0.7118		NS	
VI	IP + BOL + R	6	0.3006		NS	
VII	IP + 5-HT	5	5.5382		0.01	
VIII	IP + BOL + 5-HT	5	0.4473		NS	
I'	5-HTP	6	0.3106	0.8720	NS	NS
II'	BOL + 5-HTP	6	1.3205	2.1246	NS	0.10
III'	IP + 5-HTP	6	-	-	-	-
IV'	IP + BOL + 5-HTP	6	3.5946	2.4325	0.02	0.10
V'	BOL + R	6	3.0495	3.3601	0.05	0.02
VI'	IP + BOL	6	0.7345	1.2191	NS	NS
	5-HTP BOL + 5-HTP		1.0573	-	NS	-

weights. However, when preceded by BOL and iproniazid it caused a significant increase in adrenal weights and decrease in thymus weights in contrast to serotonin.

III. Effects on Thyroid Activity

The data in Tables 5 and 6 show the I^{131} uptake by the thyroid at 0 hour, 24 hour, and 48 hour periods, as affected by different treatments. The 0 hour value was calculated by taking the antilog of intercept 'a.' Different treatments for long term periods of forty days caused no marked differences in I^{131} accumulation by the thyroid. Further, there are no appreciable differences in the slopes. Results of the long term treatment indicate that the output rate was stabilized in all groups 24 hours after I^{131} injection. The statistical significance was not tested due to apparent absence of pronounced differences between the groups.

Although some groups of animals subjected to short term treatment of 14 days have shown a peak accumulation within 24 hours, usually the groups receiving reserpine either alone or in combination with other drugs did not reach the highest degree of thyroid radioactivity until about 48 hours. Percentage uptake was significantly higher in the group of rats receiving reserpine alone or preceded by BOL half an hour

Table 5: K'_4, K_4 and b values and zero hour, 24 hour, and 48 hour uptake of I-131, expressed as percent (%) of injected dose after chronic treatment with various drugs.

Group no.	Treatment	No. of animals	0 hour***	24 hour	48 hour	K'_4	K_4	b**
I	Reserpine(R)	3	11.510	9.800	8.780	0.129	0.146	-0.056
II	Serotonin(5-HT)	6	15.140	12.920	12.420	0.104	0.122	-0.045
III	Iproniazid(IP)	6	10.790	9.160	9.210	0.092	0.103	-0.040
IV	BOL*	4	12.220	10.340	9.870	0.124	0.142	-0.054
V	IP + R	5	9.860	8.630	8.150	0.092	0.102	-0.040
VI	IP + BOL + R	6	11.250	9.610	8.570	0.129	0.145	-0.056
VII	IP + 5-HT	6	14.960	12.820	11.320	0.140	0.165	-0.061
VIII	IP + BOL + 5-HT	6	13.400	12.020	10.190	0.120	0.138	-0.052
IX	Control	6	14.660	13.040	11.030	0.127	0.148	-0.055

*2-Bromo-D-lysergic acid diethylamide
**Semi-log slope
***The antilog of intercept 'a'. This is the theoretical maximum I^{131} uptake.

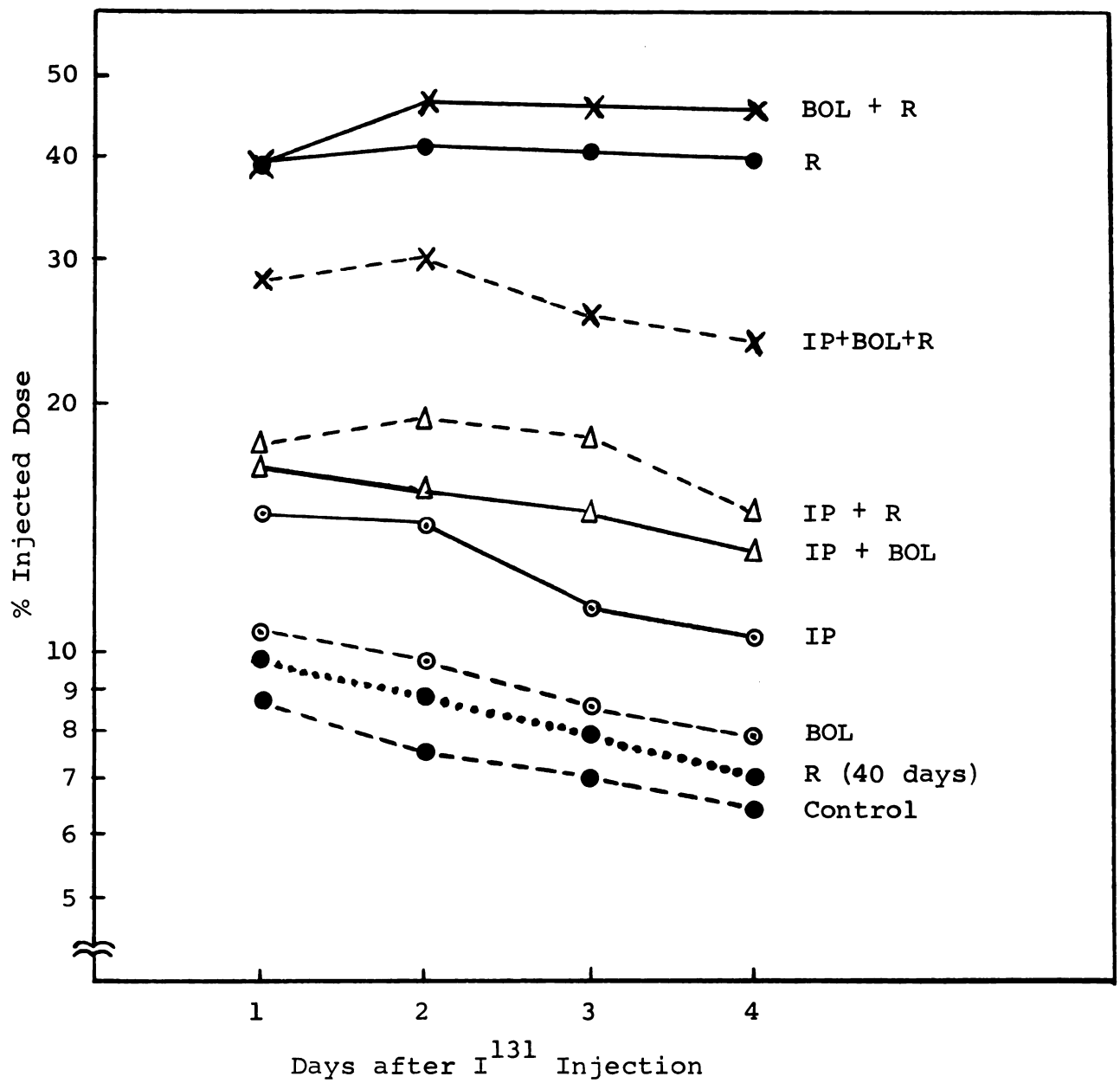


Figure 3: The thyroidal I^{131} turnover response to acute treatment with various drugs. The response to chronic treatment with reserpine is included.

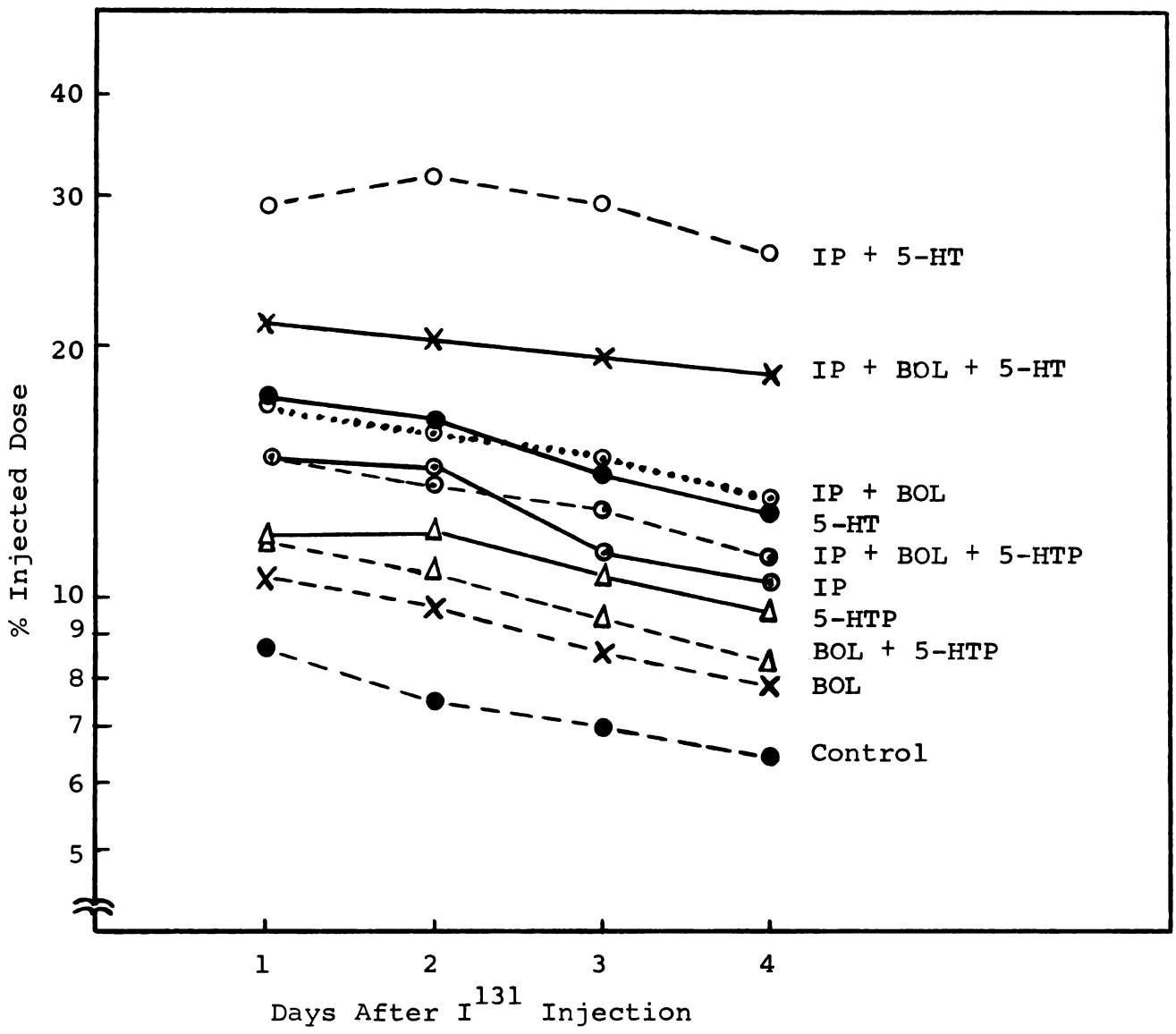


Figure 4: The thyroidal I^{131} turnover response to acute treatment with various drugs.

Table 6: b values, zero hour, 24 hour and 48 hour uptake of I^{131} expressed as percent (%) of injected dose, after acute treatment with various drugs.

Group no.	Treatment	0 hour	24 hour	48 hour	b
I	Reserpine(R)	36.570	36.260	39.573	0.009
II	Serotonin(5-HT)	21.135	18.485	17.650	-0.046
III	Iproniazid(IP)	17.240	14.713	14.320	-0.055
IV	BOL	11.683	10.580	9.738	-0.043
V	IP+R	21.848	17.743	19.165	-0.040
VI	IP+BOL+R	29.138	25.987	26.685	-0.027
VII	IP+5-HT	28.183	23.865	25.360	-0.026
VIII	IP+BOL+5-HT	22.308	20.657	18.955	-0.035
I'	5-HTP	13.071	11.802	11.798	-0.031
II'	BOL+5-HTP	13.123	11.627	10.623	-0.048
III'	IP+5-HTP	-	-	-	-
IV'	IP+BOL+5-HTP	16.158	14.670	13.580	-0.038
V'	BOL+R	41.022	40.293	46.438	0.013
VI'	IP+BOL	18.420	16.828	15.668	-0.035
VII'	CONTROL	9.446	8.727	7.518	-0.042

before reserpine injections, and the output rate was significantly lower in these groups than in others.

As seen in Figures 3 and 4 most of the groups in Experiment II have a much higher accumulation of iodine than the controls. In some cases this is very pronounced. The group of rats receiving serotonin show about two and one-half times the accumulation of I^{131} observed in the control group. The reserpine group and the group receiving BOL and reserpine indicated about four times greater accumulation of I^{131} than the control group. The pronounced variation in 24 and 48 hour uptake of I^{131} in different groups undergoing acute treatment is probably related to variations in dietary intake and excretion and the peripheral pharmacological effects of the different drugs used. Hence, no attempt has been made to derive any conclusions from the differences in the I^{131} uptake by various groups. However, the data for the experimental groups presented in Table 7 did not show any significant differences in total iodine content from the controls. A more informatory measure of thyroid function is provided by the thyroid secretion rate (TSR), shown in Table 8 and to be discussed in more detail later. The groups receiving reserpine, either alone or in combination with BOL, showed significant depression of TSR compared to the

controls, but the TSR of the other groups was not affected. These results lend further support to the view that probably the increased I^{131} uptakes by various groups is not indicative of a central effect mediated through the release of TSH. Had TSH been affected, differences in total iodide content and the output rate constant would have been found. Neither total iodide content nor iodide content per mg of thyroid was significantly different in the various groups.

The average estimated thyroid secretion rates are given in Table 8. Only the reserpine group and the group receiving BOL and reserpine showed significant and complete blockage of I^{131} output. It has been reported that lack or decrease in TSH secretion leads to both a reduced uptake and output of iodine by the thyroid. Assuming that the complete blockage of I^{131} output by the thyroid in these groups is due to reduced TSH secretion, it can be reasonably explained that the irregularity of I^{131} accumulation is due to reduced food and hence low I^{127} intake together with reduced excretion of the I^{131} administered. These factors would combine to increase the specific activity of the iodine available for thyroidal uptake and result in misleading values in the groups concerned. The pituitary-thyroid axis is known to be sensitive to slightly reduced energy intake and TSR is reduced to

maintain body weight. This may be a contributing factor (Pipes et al., 1960). However, that it is the major factor seems unlikely as the reduction in body weight and TSR seems to be a consequence of hormonal imbalance affected by reserpine action centrally.

As seen from Figure 2, iproniazid antagonizes the action of reserpine in blocking the I^{131} output. The peak of accumulation, however, is still reached after the end of 48 hours though the percent uptake is reduced. This group had a normal thyroid secretion rate and total iodine content of the thyroid. BOL did not modify the antagonism of iproniazid to reserpine action.

There were no significant variations in the thyroid weights that could be correlated to the hormonal levels.

IV. Effects on Energy Metabolism

The data presented in Tables 9 and 10 indicate the effects of long and short term treatment with various drugs and their combinations on the energy metabolism of rats. The chronic treatment with reserpine resulted in significant reduction in energy metabolism. This reduction by reserpine was blocked by prior administration of iproniazid. The maximum reduction of about 23 percent was evident after

Table 7: Effect of acute treatment with various drugs on the accumulation of iodine by the thyroid.

Group no.	Treatment	No. of rats	Thyroid wt; mg/100 gm body wt	µg I/mg thyroid	Total iodide (µg)
I	Reserpine (R)	6	6.45 ±0.71*	0.7318 ±0.1562	8.0817 ±1.37
II	Serotonin (5-HT)	6	6.21 ±0.60	0.8273 ±0.0755	13.5593 ±2.16
III	Iproniazid (IP)	4	6.87 ±1.02	0.5513 ±0.1822	7.6612 ±2.40
IV	BOL	6	6.54 ±0.46	0.7505 ±0.0748	11.4749 ±1.64
V	IP+R	4	5.49 ±0.70	0.7151 ±0.1233	10.4966 ±1.34
VI	IP+BOL+R	6	6.50 ±0.54	0.6332 ±0.0616	9.3552 ±1.16
VII	IP+5-HT	5	7.42 ±1.20	0.9075 ±0.0412	12.5939 ±2.42
VIII	IP+BOL+5-HT	5	5.94 ±0.63	0.6453 ±0.0994	10.3544 ±2.14
I'	5-HTP	6	6.65 ±0.28	0.8540 ±0.0877	15.5473 ±2.31
II'	5-HTP+BOL	6	5.34 ±0.32	0.7753 ±0.0616	12.0251 ±1.50
III'	IP+5-HTP	6	-	-	-
IV'	IP+BOL+5-HTP	6	6.00 ±0.29	0.8015 ±0.0707	11.0563 ±0.92
V'	BOL+R	6	6.07 ±0.31	0.7349 ±0.0469	10.9499 ±1.15
VI'	IP+BOL	6	6.16 ±0.42	0.6359 ±0.0812	9.5489 ±0.99
VII'	Normal Saline	6	6.09 ±0.27	0.6911 ±0.0458	11.2870 ±1.02

*Standard error of means.

Table 8: Effect of acute treatment with various drugs on the thyroid secretion rate.

Group no.	Treatment	No. of animals	Total iodide (μg)	K'_4	K_4	Thyroid secretion rate/rat	Thyroid secretion rate /100 gm rat
I	Reserpine (R)	4****	9.299 ± 1.611	-	-	0	0***
II	Serotonin (5-HT)	4	12.974 ± 3.234	0.115	0.144	3.940	1.527 ± 0.260
III	Iproniazid (IP)	4	7.661 ± 2.401	0.127	0.154	2.832	1.093 ± 0.332
IV	BOL*	4	10.176 ± 0.851	0.096	0.110	2.702	1.032 ± 0.177
V	IP + R	4	10.497 ± 1.339	0.092	0.118	2.882	1.129 ± 0.142
VI	IP + BOL + R	4	10.327 ± 1.557	0.060	0.087	2.122	0.829 ± 0.190
VII	IP + 5-HT	4	11.202 ± 2.562	0.067	0.091	2.287	1.023 ± 0.182
VIII	IP + BOL + 5-HT	4	10.364 ± 2.759	0.077	0.101	2.487	1.091 ± 0.458
I'	5-HTP	6	15.547 ± 2.31	0.071	0.083	2.916	1.069 ± 0.165
II'	BOL + 5-HTP	6	12.025 ± 1.70	0.112	0.129	3.551	1.229 ± 0.111
III'	IP + 5-HTP	6	-	-	-	-	-
IV'	IP + BOL + 5-HTP	6	11.056 ± 0.92	0.084	0.101	2.567	1.113 ± 0.199
V'	BOL + R	6	10.950 ± 1.15	-	-	0	0***
VI'	IP + BOL	6	9.549 ± 0.99	0.080	0.099	2.201	0.907 ± 0.138
VII'	Control	6	11.287 ± 1.02	0.098	0.108	2.928	1.077 ± 0.158

*2-bromo-D-lysergic acid diethylamide
**Standard error of the mean
***Groups significantly different from controls
****For facilitating the processing of data in the computer four rats from these groups were selected at random.

Table 9: The responses and changes in energy metabolism (E), treatment with various drugs.

Group no.	Treatment	Initial body wt (gms)	Initial E**	No. of animals
I	Reserpine (R)	211 ±10.67	97.32	3
II	Serotonin (5-HT)	199 ± 9.58	91.45	6
III	Iproniazid (IP)	212 ± 6.93	92.38	6
IV	BOL*	204 ± 4.56	92.07	4
V	IP + R	209 ± 6.79	91.18	5
VI	IP + BOL + R	209 ± 7.94	88.53	6
VII	IP + 5-HT	214 ± 7.57	88.05	6
VIII	IP + BOL + 5-HT	221 ± 7.67	89.66	6
IX	Control	188 ± 4.92	91.05	6

*Significantly different, at less than 0.01% level, from iproniazid plus reserpine treatment (Group V).
**Mean of three pretreatment values.
***Standard error of the mean.

expressed as percent of the pretreatment level, after chronic

% Initial energy metabolism								Body wt on Day 32
Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 32	
86.20	76.84	78.79	83.46	75.51	78.03	83.29	76.93	177*
91.08	90.78	91.09	90.79	90.29	95.16	98.48	102.12	212
94.34	91.87	92.78	94.20	98.27	94.62	103.38	101.78	226
89.94	89.21	96.13	94.27	95.20	98.52	107.49	111.86	218
92.11	95.78	89.28	89.82	92.66	88.69	91.91	90.38	212
97.84	93.02	87.18	95.89	95.14	89.21	90.42	92.42	206
94.48	99.80	93.16	97.77	94.72	98.33	110.46	103.59	197
100.32	103.09	98.76	95.34	99.84	98.00	97.97	98.10	207
108.69	110.52	100.63	114.27	107.69	105.04	104.67	106.58	229

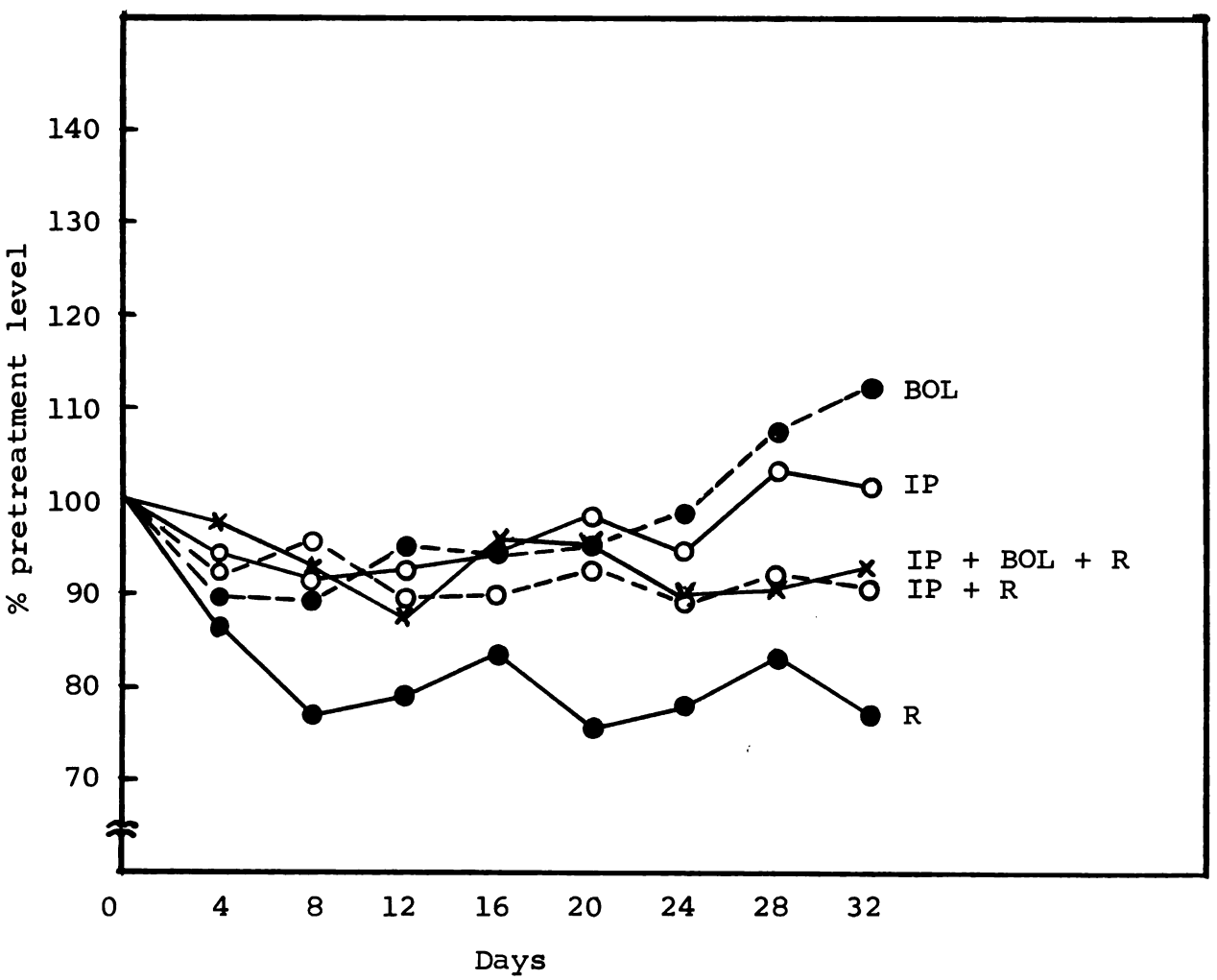


Figure 5: The response and changes in energy metabolism (E), expressed as percent of pretreatment levels, after chronic treatment with various drugs.

32 days in the reserpine group. The group receiving iproniazid prior to reserpine showed only 10 percent reduction at the end of the same period.

BOL did not reverse the blocking action of iproniazid or reserpine. Incidentally, the body weights of the reserpine group and the group receiving iproniazid plus reserpine showed a significant difference. Other groups did not show a marked change from the control values.

The acute treatment with reserpine reduced the energy metabolism by about 13 percent at the end of 8 days of treatment. However, a reduction of about 26 percent was observed at the end of the same period in chronic treatment.

It is of interest to note that iproniazid did not block the reserpine action on energy metabolism under acute treatment. On the contrary, it was further reduced to about 23 percent. The O_2 consumption, for the calculation of energy metabolism, of Groups V, VI, VII, and VIII was determined on the same day. On the 8th day, when these groups showed an abnormally low oxygen consumption, the room temperature which had so far been kept constant increased by 5^{o}. It is very likely that this sudden change in temperature led to a reduced O_2 consumption in Groups V-VIII. It is, therefore, thought that no definite conclusions can be formed regarding

Table 10: The responses and changes in energy metabolism (E), expressed as percent of the pretreatment level, after acute treatment with various drugs.

Group no.	Treatment	No. of animals	Initial body wt (gms)	Initial E***	% initial E		Body wt on Day 8 (gms)
					Day 4	Day 8	
I	Reserpine (R)	5	281	81.80	95.89	86.70	254
II	Serotonin (5-HT)	6	280	79.49	95.24	97.51	261
III	Iproniazid (IP)	6	279	84.86	98.35	100.40	256
IV	BOL**	6	283	77.85	103.64	115.44	278
V	IP + R	5	307	79.59	97.09	77.38	269
VI	IP + BOL + R	6	302	83.52	93.51	78.59	276
VII	IP + 5-HT	5	291	82.05	100.15	89.10	241
VIII	IP + BOL + 5-HT	6	288	85.68	98.83	93.21	255
I'	5-HTP*	6	285	73.69	96.59	97.61	283
II'	BOL + 5-HTP	6	300	76.33	106.38	96.27	305
III'	IP + 5-HTP	6	-	-	-	-	-
IV'	IP + BOL + 5-HTP	6	264	81.42	85.77	86.86	248
V'	BOL + R	5	304	79.41	90.71	80.93	277
VI'	IP + BOL	6	294	77.81	99.97	95.00	264
VII'	Control	6	289	82.66	95.79	94.91	283

*2-bromo-D-lysergic acid diethylamide.
**5-hydroxytryptophan.
***Mean of three pretreatment values.

the interaction of iproniazid and reserpine in acute treatment.

BOL, however, did not affect the change in energy metabolism brought about by reserpine administration.

Reserpine and serotonin are known to block the degradation of thyroxine peripherally. The state of health of the animal, nutritional factors and effect of other endocrine secretions play a part. The antagonism of thyroxine and triiodothyronine by reserpine at peripheral sites have been indicated (DeFelice et al., 1957). The results indicate the return of thyroid activity to normal after chronic treatment whereas energy metabolism continues to show a reduced level. This may be due to peripheral actions of reserpine, although the possibility of central action cannot be excluded when given in the same combination.

V. Effects on Body Weight

The effects of various treatments on body weight are shown in Table 11. Acute treatment with reserpine caused a considerable and significant decline in body weight when given alone or in combination with BOL. Chronic reserpinization caused a maximum reduction of about 29% between 16 and 20 days and acute reserpinization a decrease of about 23% on the 14th day. Thereafter, the rats gained in body

weight and after 40 days reached the initial levels. The reduction in body weight was discernible after the 4th day in both treatments and declined sharply by the 14th or 16th day.

Iproniazid when given alone or in combination with BOL blocked this decline and here again BOL had no effect on the action of iproniazid in the reduction in body weight which ensues after reserpinization.

The reserpinized animals were eating and drinking very little during the period of maximum reduction in body weight. This reduction in growth cannot be attributed only to a decreased feed and water intake. The response to reserpine, simulating stress, has only been discerned in doses which cause sedation and reduction in body weight. The animals receiving iproniazid followed by reserpine seemed to be eating and drinking normally and showed no significant decline in body weight. However, their weight was somewhat below that of the controls. Food and water intake was not determined. However, the food and water intake seems to be a contributing factor in this reduction.

From the weight curves shown in Figure 7, depression in weight was not observed to the same extent with reserpine plus iproniazid as with reserpine alone. During chronic

Table 11: The response and changes in body weight (B. Wt.), chronic treatment with various drugs.

Group no.	Treatment	Initial body wt (gm)	No. of animals	B. Wt. (gm)		
				Day 4	Day 8	Day 12
I	Reserpine (R)	211 ±10.67	3	99.54	89.81	77.78
II	Serotonin (5-HT)	199 ± 9.58	6	100.50	100.00	100.50
III	Iproniazid (IP)	212 ± 6.93	6	100.00	99.53	100.47
IV	BOL	204 ± 4.56	4	103.00	103.50	105.00
V	IP + R	209 ± 6.79	5	99.52	97.61	98.56
VI	IP + BOL + R	209 ± 7.94	6	99.04	96.65	97.61
VII	IP + 5-HT	214 ± 7.57	6	91.59	88.32	87.38
VIII	IP + BOL + 5-HT	221 ± 7.67	6	94.12	92.31	92.31
IX	Control	188 ± 4.92	6	103.19	107.45	110.11

*Standard error of the mean.

expressed as percent of initial level, after

% of initial body weight						Final body weight (gms)
Day 16	Day 20	Day 24	Day 28	Day 32	Day 40	
72.22	70.83	71.76	76.85	81.94	101.85	220 ±15.27*
100.50	102.01	102.51	103.52	106.53	112.06	223 ±13.48
100.94	101.42	103.77	105.66	106.60	110.85	235 ± 8.17
108.00	108.00	108.50	109.00	109.00	113.00	226 ± 6.67
98.56	99.04	100.00	100.00	101.44	104.78	219 ±10.63
96.65	97.13	98.09	98.09	98.56	105.26	220 ±10.08
87.38	88.32	89.25	91.12	92.06	100.47	215 ± 9.04
89.59	91.40	90.95	91.86	93.67	98.19	217 ± 9.81
113.30	115.43	118.09	119.68	121.81	127.13	239 ±12.97

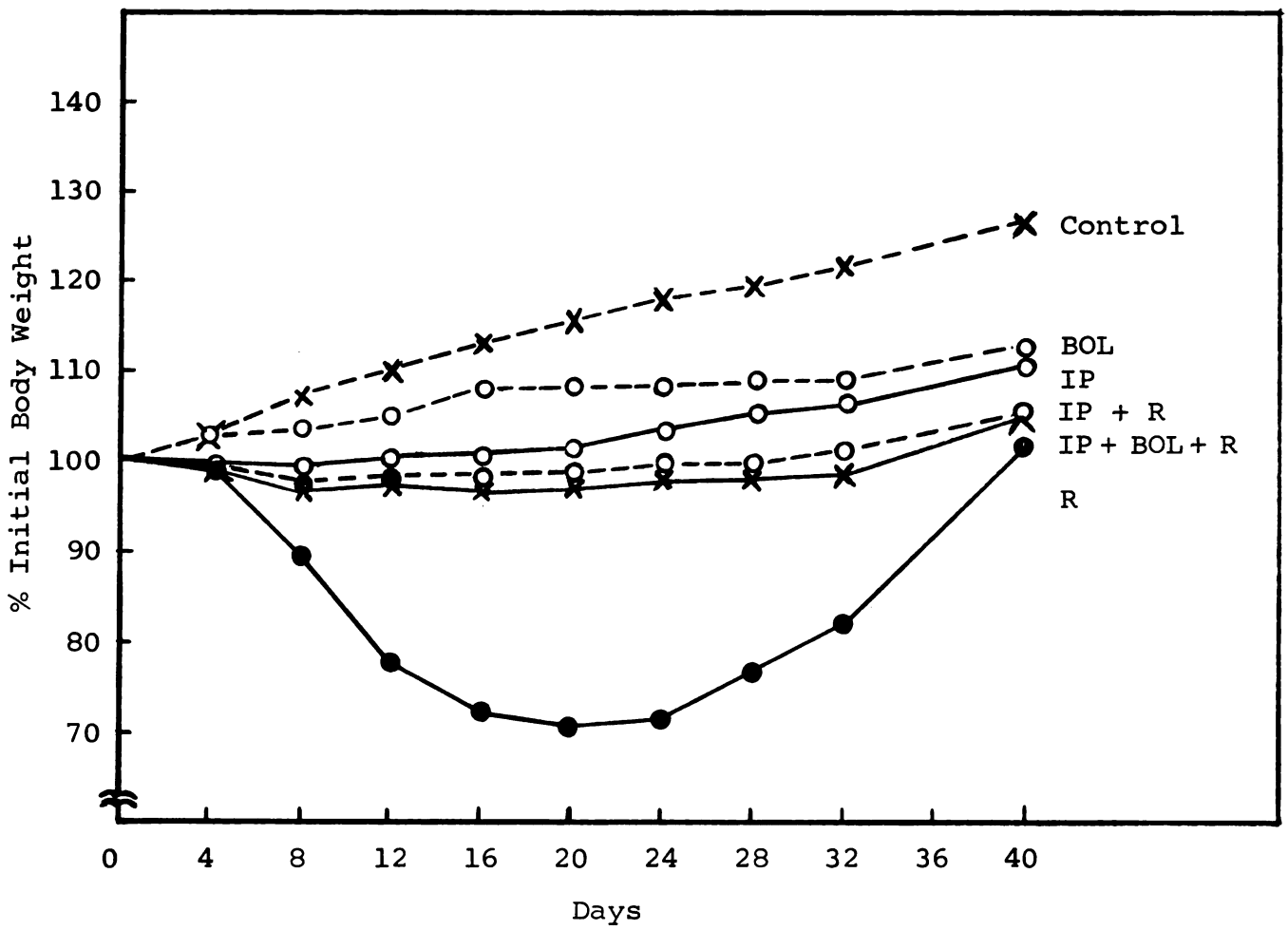


Figure 6: The response and changes in body weight (B.Wt.), expressed as percent of the initial level, after chronic treatment with various drugs.

Table 12: The response and changes in body weight (B.Wt.), expressed as percent of of initial level, after acute treatment with various drugs.

Group no.	Treatment	No. of animals	Initial body wt (gm)	% of initial body wt.			Final body wt
				Day 4	Day 8	Day 14	
I	Reserpine	6	281 ± 6.51*	99.15	89.65	77.06	217 ± 5.69*
II	Serotonin	6	280 ± 6.01	97.85	93.48	92.35	258 ± 6.91
III	Iproniazid	6	279 ± 12.70	96.65	91.63	87.31	243 ± 9.46
IV	BOL	6	283 ± 6.17	100.77	98.33	93.28	264 ± 6.01
V	IP + R	6	307 ± 11.91	96.41	90.95	87.00	267 ± 11.03
VI	IP + BOL + R	6	302 ± 9.92	96.23	91.60	85.39	258 ± 7.70
VII	IP + 5-HT	5	291 ± 4.56	92.20	84.44	77.66	222 ± 2.46
VIII	IP + BOL + 5-HT	6	288 ± 6.92	93.71	88.69	81.77	235 ± 7.29
I'	5-HTP	6	281 ± 4.03	99.17	100.60	96.80	272 ± 4.12
II'	BOL + 5-HTP	6	297 ± 1.42	100.28	102.58	97.12	289 ± 2.41
III'	IP + 5-HTP	6	Died	-	-	-	-
IV'	IP + BOL + 5-HTP	6	259 ± 5.20	96.26	94.34	90.19	234 ± 6.11
V'	BOL + R	6	295 ± 8.00	100.82	93.15	79.54	234 ± 6.68
VI'	IP + BOL	6	286 ± 11.44	96.34	92.31	86.08	246 ± 10.24
VII'	Control	6	281 ± 9.25	101.84	100.77	96.84	272 ± 9.78

*Standard error of the mean.

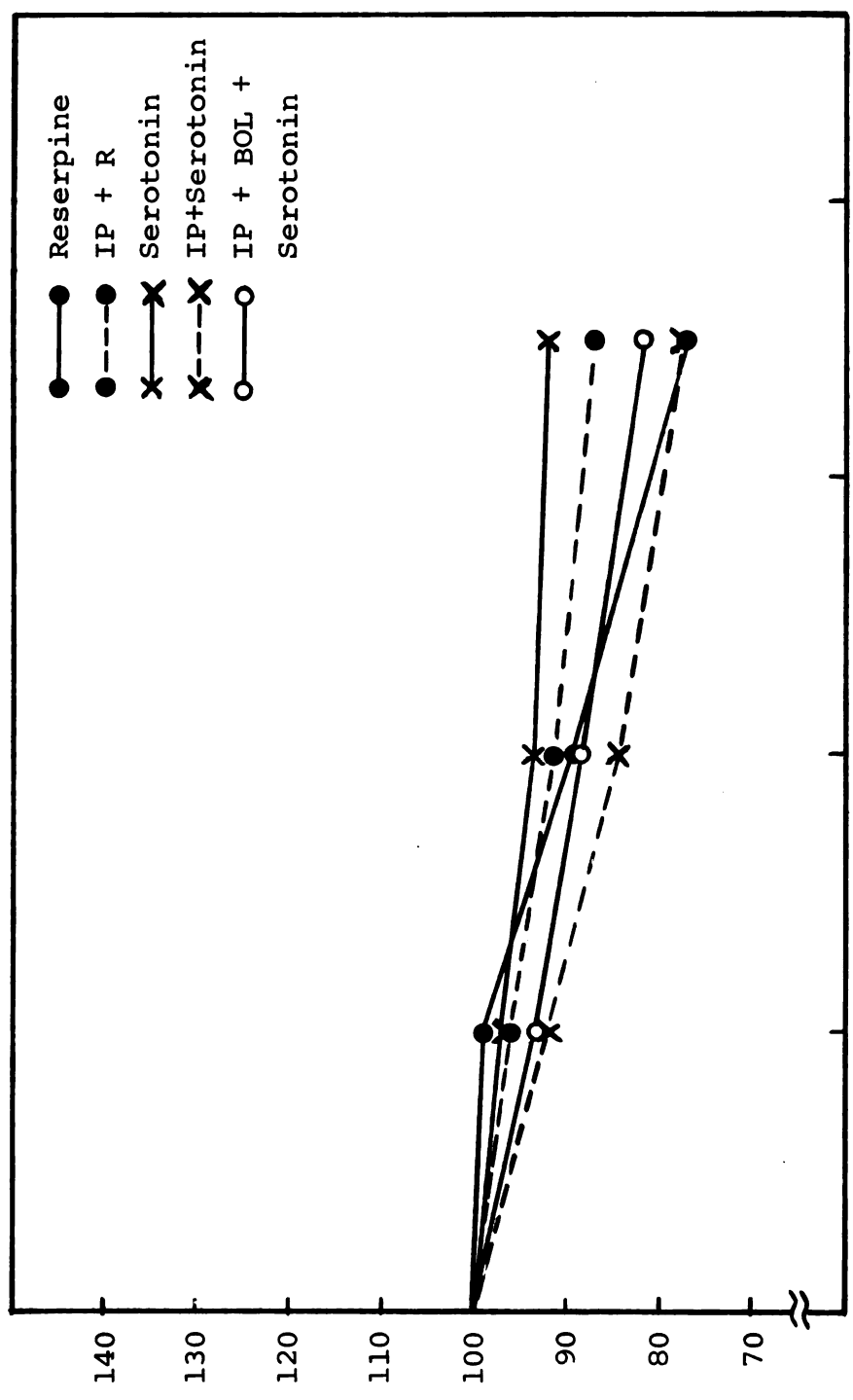


Figure 7: The response and changes in body weight (B.Wt.), expressed as a percent of initial level, after acute treatment with various drugs.

treatment with serotonin, initial body weight was maintained whereas under acute treatment about 8% reduction was observed. Pretreatment with iproniazid accentuated the effects of serotonin bringing the maximum reduction to about 13% between the 12th and 16th days under chronic treatment and to about 23% in acute treatment of 14 day. BOL tended to antagonize these effects of serotonin though not very effectively. After the administration of BOL with iproniazid and serotonin, in chronic treatment, the maximum reduction was brought down to about 11%, and in acute treatment to about 19%.

DISCUSSION

Many attempts have been made to identify the substances involved in central neuronal transmission. Serotonin and norepinephrine have long been thought to have a certain role in this regard. There seems to be no doubt about the release of these substances both centrally and peripherally after reserpine administration. Disturbances caused by tranquilizers are presumed to result from their action on the diencephalon and particularly the hypothalamus (Smelik and Sawyer, 1962). However, attempts to correlate reserpine actions to serotonin or norepinephrine have not yielded clear concepts, either concerning the mechanism of serotonin and norepinephrine actions or the neuroendocrine mechanism in which these are thought to be involved. The interest in serotonin has been more widespread but no one has succeeded in establishing an unequivocal role for serotonin in normal physiology or in mental disorder.

The results presented here indicate that the response to reserpine simulated stress during the first part of the chronic treatment and throughout the acute treatment, showing a sustained action on neuronal pathways in the brain as suggested by Costa et al. (1962). Responses to stress and

reserpine have been indistinguishable. Reserpine depresses the response of the rat to acute stress by inhibiting the hypothalamic centers (Sevy et al., 1957). Several investigators have suggested that the central effects of reserpine are mediated through the parasympathetic influence in the hypothalamus and a depression of central sympathetic structures in the diencephalon.

Usually parasympathetic effects of reserpine have been associated with a low concentration of free serotonin in brain whereas with a high concentration sympathetic predominance results. However the recent findings of Costa et al. (1962) do not substantiate this. Administration of α-methyl-m-tyrosine (α-MMT) depletes brain norepinephrine and not serotonin. They found that after depletion of catecholamine stores in CNS with α-MMT sedation and other signs of reserpine are not evident. Subsequent administration of reserpine induced sedation usually seen after reserpine. It is difficult to reconcile the fact pointed out by their study and derive any correlation between reserpine and α-MMT actions unless the sedative effects of reserpine are induced by the peripheral autonomic imbalance resulting from depletion of peripheral amines. Their results tend to indicate that the presence of serotonin and absence of norepinephrine does not lead to

central activity, whereas, the excitation ensuing after the administration of iproniazid and reserpine is generally attributed to excess of norepinephrine, most likely peripherilly (Shore, 1962). This would indicate that it is the increase in peripheral stores of norepinephrine after the administration of iproniazid and reserpine that either induces excitement or reverses the reserpine effect. Further, the depletion of norepinephrine centrally, has no effect on the central activity and other reserpine like effects even when serotonin is present. In that the release of norepinephrine by α-MMT had no reserpine-like effects, whereas, the sympatholytic effects of reserpine are attributed more to the drug-induced absence of peripheral sympathetic mediator, norephinephrine, further studies are warranted. However, this indicates that serotonin in some way correlated with reserpine action, at least in inducing adrenal changes.

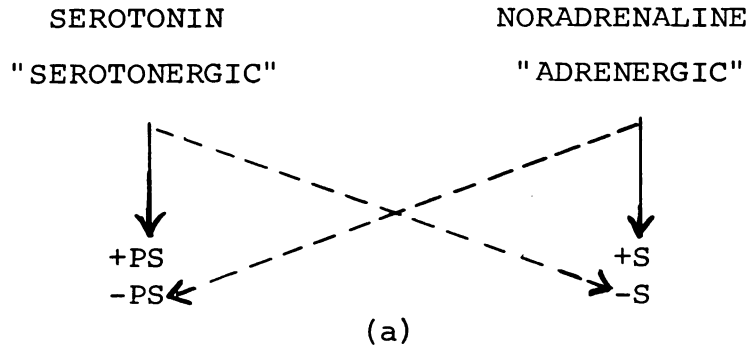
The studies on uptake of norepinephrine by the CNS after α-MMT administration and its effect on peripheral stores of catecholamines, including the adrenal medulla, would be important. More so, because reserpine is known to induce the depletion of central and peripheral norephinephrine stores and also block its uptake (Shore, 1962).

The results presented here show that the administration of iproniazid two hours before reserpine injection blocked the effect of reserpine on sedation. Generally such a treatment has been reported to lead to a central excitation (Brodie et al., 1956; Chessin et al., 1957). Shore et al. (1957) attributed the central effects to high levels of serotonin in the brain due to the prevention of destruction of serotonin (Spector et al., 1960). On the contrary, the present study did not show any visible signs of excitation and the rats appeared to be behaving normally. It seems unlikely that the sedative action (parasympathetic) of reserpine is mediated through the release of serotonin, and excitation (a sympathetic effect) is due to maintenance of high levels of serotonin for a long period after treatment with iproniazid. The failure of BOL, to reverse the effects of iproniazid plus reserpine, and thereby cause sedation, indicates that serotonin released by reserpine might not be involved in its sedative action. The present studies show that BOL did not antagonize the reserpine effects. This would be in keeping with the fact that serotonin is not likely to be present in large amounts after reserpine administration as the serotonin released is rapidly degraded to its inactive metabolite- 5-HIAA. Hence no serotonin would be

available to cause a deep sedation, particularly after prolonged treatment. Sedation was not observed until after four days of treatment with reserpine. During this period the serotonin stores in the body, particularly in the more sensitive brain, would be depleted.

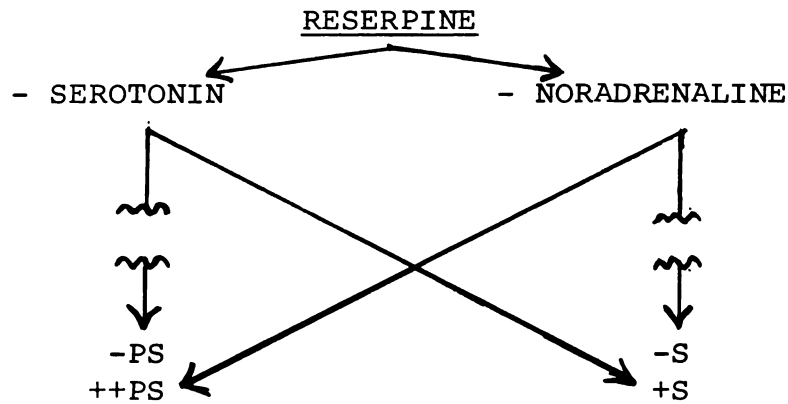
Chlorpromazine, probably by depressing the hypothalamic sympathetic centers (Shepherd and Wing, 1962), removes the sympathetic influence causing parasympathetic predominance and sedation. It does not cause the release of serotonin (Carlsson, 1957). In dogs and cats the levels of serotonin are raised by reserpine up to ten times the normal in the CNS after pretreatment with iproniazid, with iproniazid, without causing pronounced excitation. However, the adrenergic response in reserpinized rats pretreated with iproniazid has been correlated to the increased levels of norepinephrine rather than to serotonin (Franco Browder, 1958; Spector et al., 1959). It appears, therefore, that MAO might control the amount of biogenic amines of the CNS inside the neuron. It has been maintained that the MAO inhibitors do not simply stabilize the freed amines but actually prevent the release of free amines (Shore, 1962). In addition it is probable that these or some other unknown amines are

engaged in maintaining a constant balance between parasympathetic and sympathetic systems. A likely explanation for the excitement observed in rats by other workers after a combined treatment of iproniazid and reserpine can be a sympathetic dominance due to raised levels of norepinephrine. An alternated possibility suggested on the basis of the present results is that normally serotonin leads to stimulation of the parasympathetic structure and norepinephrine to the sympathetic structure in the CNS. Reserpine leads to the depletion of both serotonin and norepinephrine stores in the CNS and peripherally resulting in a severe imbalance of the peripheral and hypothalamic autonomic systems. The net result of such an imbalance is a parasympathetic dominance. This parasympathetic effect seems more likely to be related to the absence of sympathetic influence induced by reduced adrenergic amine than to a release and/or depletion of serotonin levels. The released serotonin is not likely to persist in the presence of uninhibited MAO. In view of this it is difficult to understand why the parasympathetic effect following reserpine administration is commonly attributed to the release of serotonin (Fig. 8 shows some alterative views in this respect.). Thus in the presence of persistent high levels of serotonin and norepinephrine in reserpinized animals pretreated with iproniazid any of the following effects can



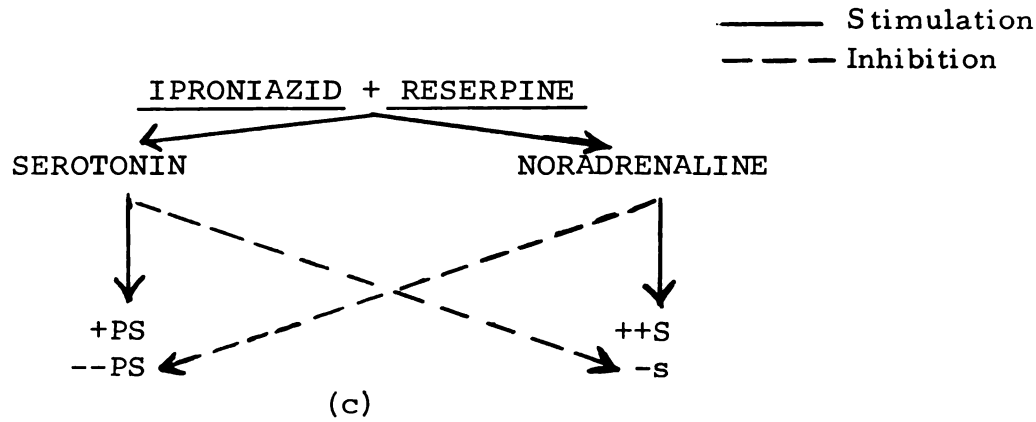
(a)

Under normal conditions a balance is maintained between parasympathetic and sympathetic functions.



(b)

Under such conditions the net result seems to be a parasympathetic predominance mainly due to the release from sympathetic influence.



(c)

Under these conditions either (1) a normal balance is maintained or (2) sympathetic predominance ensues leading to excitation.

Figure 8: A scheme showing the reciprocal relationship between sympathetic and parasympathetic structures in the hypothalamus.

be observed depending on the level of sympathetic and parasympathetic activity:

(1) Due to accumulation of sympathomimetic amines the animals may show sympathetic dominance and hence excitation.

(2) A normal balance between sympathetic and parasympathetic systems may prevail without eliciting any overt signs of excitation.

Rats receiving reserpine on a long term basis showed recovery after about twenty days of sedation and reduced dietary intake. This observation may be related to reduced or no ACTH secretion in response to chronic treatment with reserpine as will be discussed in a later section. This may also be attributed to an adaptation to the drug action.

It is difficult to interpret the disparity in the sedative action of serotonin between two experiments in view of the fact that serotonin is not known to enter the blood-brain barrier or if it enters at all it is with great difficulty (Costa and Aprison, 1958). However, sedation is a central action. Serotonin is reported to potentiate hexobarbital hypnosis (Page, 1954). In the present study pretreatment with iproniazid, in acute treatment, further accentuated the serotonin effect on sedation. This sedation was more deep than that found in reserpine-treated rats.

The rats were unresponsive to external stimuli like noise and pinching of toes. Toxic doses of serotonin cause paresis of the hind limbs (Page, 1954). It seems probable, however, that these actions of serotonin are mediated through peripheral effects. Direct evidence of the influence of serotonin on vagal afferent impulses in rats has been observed from study of the stretch receptors in the lungs, and injection of serotonin may stop respiration in the expiratory phase (Page, 1954). Sensory nerve endings may be involved as well. In man intravenous administration of serotonin causes a generalized numbness. Such numbness was observed in the present study too. BOL antagonized these effects to a marked extent. However, BOL does not appear to reverse the iproniazid effect on reserpine. This indicates the probability that either BOL does not enter the CNS or else the blocking effect of iproniazid on reserpine action is not due to accumulation of serotonin. That BOL does not modify appreciably the effects of iproniazid on reserpine action on the energy metabolism, body weight, thyroid and adrenal activities indicates the probability of BOL not being able to enter the CNS. This is contrary to the belief of Cerletti and Rothlin (1955). However, as shown in the results presented here BOL was observed to antagonize some serotonin

actions which can be considered as its peripheral pharmacological effects. These include the adrenal hypertrophy and thickening of the dermis after administering iproniazid and serotonin. These results, therefore, indicate the possibility that BOL is less likely to enter the CNS and that it antagonizes only the peripheral actions of free serotonin.

The deaths of rats receiving iproniazid two hours before 5-HTP can be similarly explained. They may be mainly due to to the peripheral pharmacological action of serotonin, probably a respiratory failure. Respiratory failure can be provoked by high doses of 5-HTP particularly when given in anaesthetized rats and other animals (Erspamer, 1954). The fact that rats died within about six hours of the injection of 5-HTP indicates a peripheral involvement. BOL again antagonized these effects of 5-HTP. The group of rats receiving iproniazid, BOL and 5-HTP survived for the duration of the acute treatment. However, if the possibility that these actions may be mediated centrally is not excluded, the assumption that BOL and serotonin may enter the CNS would have to be made. Results described earlier indicate the hypertrophy of adrenals in the group of rats receiving iproniazid, BOL and 5-HTP. This hypertrophy of the adrenals may indicate the antagonism by BOL of the serotonin action with

respect to ACTH activity. It might appear that the hypertrophy is not a resultant of the direct effects of serotonin, biotransformed from 5-HTP, on the adrenals. This might be indicated by the fact that BOL did not block the hypertrophy as effectively as it did in the case of combined treatment with iproniazid and serotonin, both under acute and chronic treatment. It is hypothesized in the later part of this discussion that serotonin normally inhibits the release of ACTH. If the assumption that BOL enters the CNS is correct then the effect of BOL could be postulated to be as a result of serotonin antagonism within the CNS. BOL did show an antagonism to the combined action of 5-HTP and iproniazid on mortality, which, as discussed in the last section may be explained to a large degree on the basis of peripheral mediation. However, it is very likely that, in this particular treatment the dose of BOL is not high enough to antagonize the effects of large amounts of serotonin (biotransformed from 5-HTP and protected by iproniazid) on adrenal cortex, thus excluding the possibility of BOL entering the CNS. The involvement of CNS in these actions is discussed further in a later section.

The release of ACTH has been reported both under stressful conditions and after reserpine administration (Kitay et

al., 1959; Saffran and Vogt, 1960). Under acute treatment reserpine administration led to significant hypertrophy of adrenals, probably due to the persistent and prolonged discharge of ACTH from the pituitary as observed by Montanari and Stockham (1962). Westermann (1962) showed a close association between ACTH hypersecretion and the blockage of serotonin storage in the brain. They postulated that the hypersecretion of ACTH results from the action of reserpine on neuronal pathways that monitor the anterior pituitary. "Serotonergic" fibres have been postulated to be present in the hypothalamus (Heller, 1962). Chronic treatment with reserpine in the present study, is in confirmation of the results of Khazan et al. (1961) who observed the "normalization" of adrenals after chronic treatment with reserpine. They observed the maximum effect on adrenal hypertrophy after ten days treatment. Maickel (1961) showed that reserpine causes ACTH release only when given in doses which induce sedation and loss in body weight. During chronic treatment, probably, the pituitary either becomes adapted to the drug action as suggested by Wells et al. (1956) or there is not enough ACTH to cause adrenal stimulation (Maickel et al., 1961). The release of this hormone appears not be be balanced by its resynthesis. The resulting fall

in pituitary stores of ACTH can generally be used as an index of increased secretion. On the basis of these works and the findings of the present study it seems likely that conditions simulating stress after reserpine administration may be due to excess ACTH secretion. Depletion of ACTH stores probably leads to gradual normalization of the rats with respect to the central sedative action, growth and adrenal weights. Reserpine is not known to be localized in specific parts of the CNS (Sheppard et al., 1957). It may block the transmission of the afferent impulses to the CNS (Schneider et al., 1955). The possibility that the CNS becomes adapted to the drug action, thereby establishing a new level of parasympathetic-sympathetic balance, cannot be excluded. The present findings indicate that pretreatment with iproniazid blocks the adrenal hypertrophy which ensued in response to acute treatment with reserpine alone. BOL did not reverse this iproniazid action. This fact argues against the possibilities of serotonin being involved directly in the manifestation of reserpine action as proposed by several investigators. There is no convincing evidence to support this wide spread assumption. On the contrary the results presented here indicate that serotonin normally might have an inhibitory role in the neurohumoral transmission in the CNS (Figure 9).

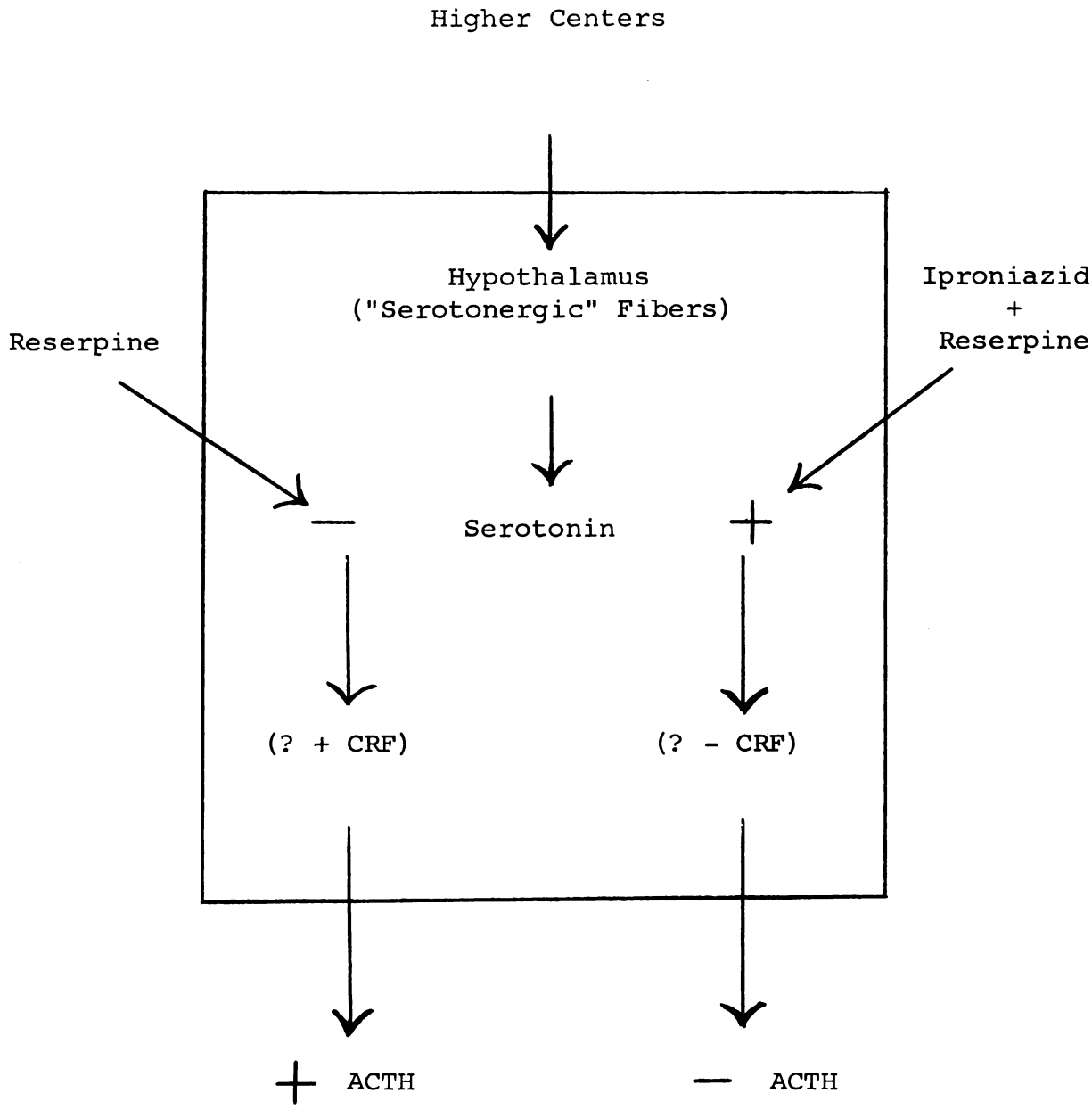


Figure 9: The inhibitory role of serotonin in the hypothalamus. The site or sites of action of reserpine and iproniazid are not clear.

Control of ACTH secretion by hypothalamic fibres liberating a humoral substance into the hypophyseal circulation has been suggested by Maickel et al., 1961, Westermann et al., 1962, Montanari and Stockham, 1962. This agent is probably CRF - a polypeptide. The secretion of ACTH may be controlled by both inhibitory and stimulatory pathways in the hypothalamus as suggested by Egdahl (1960). Control of ACTH secretion by such a system is represented in Fig. 9. It seems likely that the stimulation of ACTH secretion by reserpine results from both the depression of inhibitory pathways (due to depletion of serotonin) and the transitory release of epinephrine in big amounts thus activating stimulatory pathways. Thus, the present findings as well as reports by other workers can be explained on the basis of assigning a normal inhibitory role to serotonin in the release of ACTH, probably through the inhibition of CRF. Both CRF and serotonin are found in the hypothalamus. It is of interest to mention the results of Costa et al. (1962). They were interested to find whether discharge of ACTH after reserpine administration is related to changes in brain norepinephrine or in serotonin. They found no release of ACTH after administration of α-MMT. Subsequent administration of reserpine induced adrenal changes usually

seen after reserpine. This would be in keeping with the hypothesis presented here (Fig. 9) based on results of the present study that serotonin usually has an inhibitory role in the hypothalamus. There is also evidence for the presence of "serotonergic" fibers in the hypothalamus. Consequent to reserpine administration there is persistent reduction in serotonin content in the hypothalamus due to its rapid release and destruction by MAO. This in turn results in a depression of inhibitory pathways persistently causing uninhibited ACTH release. These inhibitory pathways may involve "serotonergic" fibres in the hypothalamus as postulated by Brodie (1957) and Heller (1962). It has already been pointed out that reserpine administration is followed by parasympathetic dominance. Such a hypothesis is strengthened by the evidence that reversal of reserpine action ensues if iproniazid is injected prior to reserpine injection. Such a reversal of reserpine action has been reported not to ensue if iproniazid is given simultaneously or after reserpine administration, as serotonin is rapidly released by reserpine and metabolized by uninhibited MAO. If this hypothesis is correct it would be expected that the continued presence of relatively excess serotonin should cause reduction in ACTH activity. Indeed the present results

indicate reduced adrenal weights in groups of rats receiving iproniazid followed by reserpine. This reduction was significant as compared to the effects of reserpine alone. However, the adrenal weights were not significantly different from the controls. BOL had no effect on the concomitant action of iproniazid and reserpine. The possible mechanism of action of BOL has already been discussed. The dosage cannot be questioned as BOL in the doses given has been shown to be very effective in antagonizing certain actions assigned to serotonin in this study but for the doubts expressed earlier in case of group of rats receiving 5-HTP and iproniazid. Further, the absence of any antagonism by BOL of any of the reserpine actions in this study indicates that most of the reserpine actions except that on ACTH release are not mediated through the release of serotonin in the body. It is likely that there would be little serotonin for BOL to antagonize after reserpine injections. However, BOL is absorbed rapidly and was injected a half-hour before reserpine.

These results further suggest that serotonin may have different roles centrally and peripherally. In fact serotonin might not have any normal physiological role peripherally and all its peripheral actions might just be the pharmacological effect of the drug <u>per se</u>. The mechanism of concentration

of serotonin in platelets and brain is different. Platelets can concentrate serotonin solely by an active transport mechanism whereas brain does so through the uptake of 5-HTP (Shore, 1962). Plasma has insignificant amounts of serotonin. The other major storage site for serotonin is gastrointestinal tract. It may well be that platelets are used for transport of released serotonin from brain and the gastrointestinal tract for the uptake of tryptophan from the diet for eventual biotransformation to serotonin. However, the possibility is strong that serotonin may have a hormonal role in the mediation of parasympathetic effects, similar to the role of noradrenaline in the mediation of peripheral sympathetic effects. These views lend support for the hypothesis that the major role of serotonin might be in the brain.

A direct action of serotonin on the adrenal cortex has been demonstrated by Rosenkrantz and Laferte (1960) and Verdisca et al. (1961). Chronic or acute treatment by serotonin did not cause any significant change in adrenal weights as seen from the results presented here. This was probably due to rapid metabolism of administered serotonin by MAO. Pretreatment with iproniazid caused a significant hypertrophy of adrenals under both treatments. This action

can be advanced as evidence of a direct action of serotonin on adrenals not mediated through the release of ACTH. It is worthwhile to note that iproniazid here potentiated the effects of serotonin but had antagonized the action of reserpine as far as adrenal weights are concerned. BOL effectively and significantly antagonized this sustained action of serotonin on the adrenal cortex.

5-HTP causes an immediate increase in central serotonin levels. Rats receiving iproniazid, BOL and 5-HTP showed a hypertrophy of adrenals in contrast to a group of rats receiving iproniazid, BOL and serotonin. Three possibilities can be advanced for this action.

(1) BOL antagonized the inhibitory action of serotonin, in the CNS, on the release of ACTH thereby causing the same effect on adrenals as reserpine.

(2) A likely acetylcholine-like action of serotonin when present in excess. Acetylcholine when present in excess inhibits its own action (Burn and Rand, 1962). Thus a sudden increase in serotonin levels in the brain may simulate acetylcholine-like action by suppressing its own inhibitory role in ACTH release.

(3) In this particular case, in presence of excessive and sudden rise in levels of serotonin, the dose of BOL was

not high enough to antagonize serotonin action.

The first possibility does not seem to be likely in view of the prior discussion.

Reciprocal inhibition of the anterior and posterior hypothalamic zones has been shown in cats (Gellhorn, et al., 1956). It has been indicated that at the more caudal end of the hypothalamic site facilitory and inhibitory areas are reciprocally innervated for pituitary-adrenal response (Slusher and Critchlow (1959). The activities of one inhibit the activities of others. The anterior basal part of the hypothalamus has been cited to be essential for the maintenance of TSH release. Gellhorn et al. (1956) found that lesions of the anterior hypothalamus produced release of posterior sympathetic functions while posterior hypothalamic lesions potentiated anterior parasympathetic functions. Thus the assumptions so far made, lead to the view that stimulation of the parasympathetic neural system, represented to some extent in the anterior and lateral hypothalamus, inhibits the activity of the sympathetic system and more generally these two systems are reciprocally innervated. The adjoining diagram (Fig. 10) shows the location of hypothalamic sites believed to be monitoring the adenohypophyseal hormones.

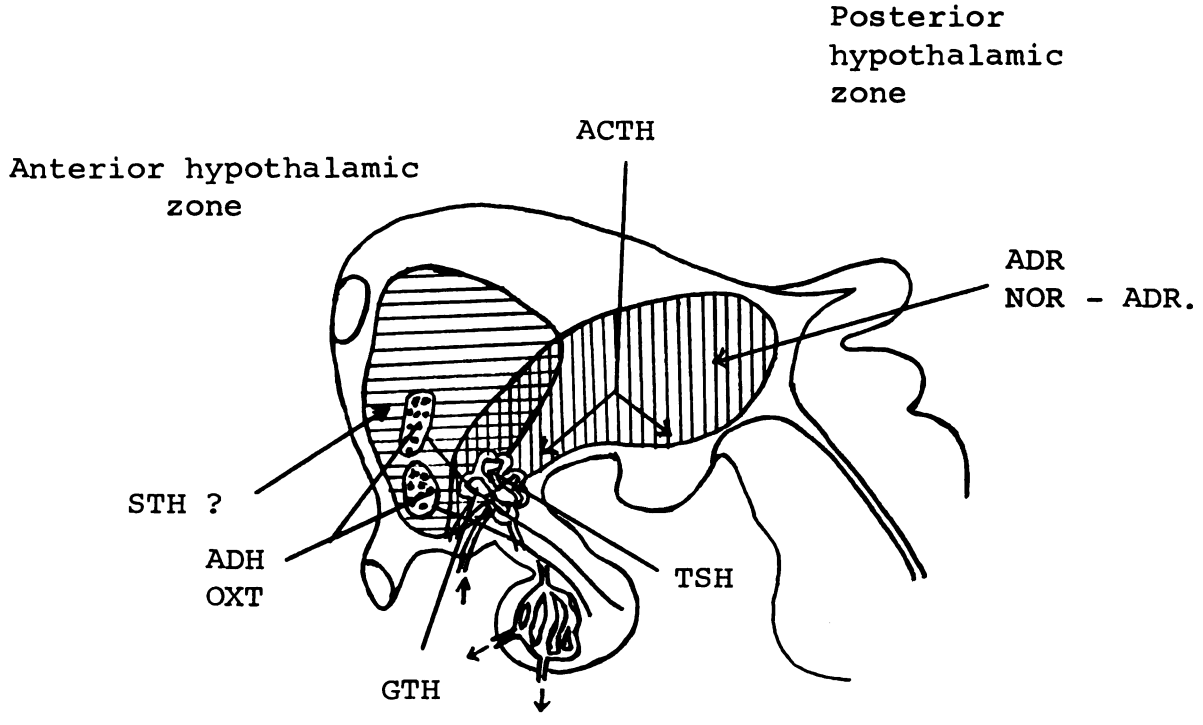


Figure 10: The hypothalamus and pituitary in median sagittal section showing the hypothalamic sites found to be associated with hormonal release into the hypothalamo-adenohypophyseal portal vessels (Bovard, 1961).

The results presented here are in agreement with the hypothesis presented on the previous page. Reserpine alone or in combination with BOL under acute treatment showed an increase in adrenal activity with a simultaneous decrease in thyroid activity. The complete inhibition of output of radioiodine by rat thyroid receiving acute treatment with reserpine or reserpine and BOL (Fig. 2) is indicative of reduced TSH secretion. Reduced thyroid activity has been postulated by many workers to result from decreased TSH secretion (Moon and Turner, 1959a, 1959b). Reduced TSH activity should also cause a reduction in uptake of radioiodine as shown by Moon and Turner (1959a, 1959b). The present results indicate a very high uptake of radioiodine by acutely reserpinized rats as compared to controls. This might be misleading. However, it should be noted that after the fourth day of drug treatment the rats were having very little dietary and water intake. This, probably, resulted in the uptake of radioiodine of high specific activity. This high level was maintained due to reduced excretion of I^{131} and lowered intake of dietry I^{127} . Had this increase in the uptake of radioiodine been in response to increased TSH activity there would be expected a higher total iodine content of the thyroid and a greater thyroid secretion rate

(TSR). On the contrary the results indicate no significant difference in total iodine content of the rat thyroid of the groups receiving reserpine alone or in combination with BOL. No evidence for thyroid secretion was observed as seen from the slope and TSR values. It seems likely that the reduction of TSR reflects a decrease in TSH secretion. Iproniazid reversed this reaction of reserpine both in the amount of radioiodine uptake and TSR. The slope and TSR were not significantly different from the controls. BOL had no effect when given with iproniazid and reserpine.

The effects on thyroid activity following iproniazid plus BOL and reserpine are not conclusive. These groups and the groups receiving iproniazid and serotonin or iproniazid, serotonin and BOL showed higher zero hour, 24 hour and 48 hour uptakes. These divergent effects may be due to the differences in responses to these drugs. However, the TSR and total iodine content of the thyroid were not significantly different from the controls. It seems worthwhile to mention here that BOL when given with iproniazid and serotonin reduced the 24 hour uptake from about 30% of the injected dose to about 20% and the peak accumulation was reached within 24 hours in contrast to 48 hours in case of iproniazid and serotonin. On the other hand the administration of BOL with

iproniazid and reserpine increased the uptake from about 20% to about 30% of the injected dose.

These results point out the involvement of a neurohumor or neurohumors in maintaining a balanced neuroendocrine function. Parasympathetic and sympathetic balance seems to be important in maintaining such a homeostasis. The possibility of one or more neurohumors being involved in controlling the neuroendocrine functions, either directly or through the autonomic system, is very likely.

At present serotonin and norepinephrine seem to be the most likely neurohumoral transmitters in the brain. However, their mechanism of action and the degree of involvement in CNS function is not clear. Vogt (cited by Crossland, 1960) has commented that "our ignorance as regards the function of brain sympathin could not be more complete," whereas, concerning the role of serotonin as a central transmitter, Crossland (1960) has pointed out that the known facts ". . . allow experimental evidence to be fitted into almost any theory the investigator chooses."

The results presented here show that the chronic treatment with reserpine caused a steep reduction in energy metabolism after the fourth day of treatment. Iproniazid blocked this reduction and maintained the metabolism at basal levels.

However, the reserpine group did not show a return to the initial level after 32 days even though the body weight and the thyroid activity showed a recovery. This might be due to the peripheral antagonism by reserpine on the action of thyroxine and triiodothyronine on oxygen consumption as shown by Kuschke (1954) and De Felice (1957). Acute treatment with reserpine and BOL plus reserpine also resulted in a reduced level of energy metabolism. However, it was not antagonized by iproniazid. No conclusion could be come at for this disparity as on the eighth day of the measurement of oxygen consumption the groups receiving not only iproniazid and reserpine but also those receiving iproniazid plus BOL and reserpine, iproniazid and serotonin, and iproniazid plus BOL and serotonin showed a marked decrease in oxygen consumption probably due to increase in room temperature by five degrees. Although other treatments did not show a marked change in energy metabolism, an initial reduction was followed by a tendency towards recovery. The effects on energy metabolism, probably are due to the peripheral pharmacological effects and do not indicate a pattern involving central nervous system.

The chronic treatment with reserpine induced a precipituous loss in body weight. The rats showed a trend to

recover after 20 days of treatment and reached the initial levels after 40 days. Montanari and Stockham (1962) showed that reserpine caused ACTH release only when given in doses inducing sedation and loss in body weight. Acute treatment with reserpine or with BOL and reserpine also induced loss in body weight. This indicates that loss in body weight after reserpine administration is a consequence of "stress" produced by ACTH release and not the principal action of the drug. The reduced dietary intake would greatly effect the body weight. That this reduced dietary intake is in response to "stress" and not the direct effect of the drug should be noted. Iproniazid prevented this reduction in body weight effectively although the rats receiving iproniazid and reserpine did not grow at the same rate as the controls. Chronic treatment with other drugs induced initial loss with subsequent recovery. Acute treatment induced a loss in body weight but not to the same extent as seen after administration of reserpine. However, acute treatment with iproniazid and serotonin induced a loss in body weight equivalent to that of reserpine. The groups of rats subjected to acute treatment were older. It would be expected that they would either maintain their body weight or show a decrease.

The effects of these agents on body weight indicate a peripheral pharmacological effect due to the differences in response to various drugs and are not very conclusive. It is important to mention that a treatment with reserpine showed a maximum loss which was antagonized by iproniazid to an appreciable extent.

These results indicate that reduction in body weight and energy metabolism is mainly a consequence of the stress produced by the release of ACTH by reserpine. The peripheral pharmacological effects also play an important role.

It would be rather naive to hope that this study and its interpretation might reveal dramatic insights into the mechanism of reserpine and serotonin actions or in the understanding of certain neuroendocrine mechanisms these agents were used for. However, as Bogdanove (1962) observed "concepts based on inadequate evidence are fantasy, but it is often from efforts to test such fantasy that new facts emerge." This problem may perhaps have no solution for a long time. Much work will have to be done before the final role of these substances is established. It is believed that efforts to test the hypothesis presented here, even though it might appear a fantasy, would be worthwhile.

SUMMARY AND CONCLUSIONS

The interactions of reserpine, serotonin and related drugs on the autonomic nervous system, adrenal gland, and thyroid function, energy metabolism and body weight were studied.

1. Acute reserpinization for 14 days induced sedation. The reserpine action was slow in onset. Chronic reserpinization also resulted in sedation during the first half of treatment. This was followed by recovery by the 40th day. Rats receiving iproniazid before reserpine were resistant to the depressant effects of the latter drug. Neither the concomitant administration of BOL and reserpine showed any deviation from the aforesaid reserpine effect nor did BOL reverse the "normalization" seen in the reserpinized rats pretreated with iproniazid.

Acute but not chronic treatment with serotonin resulted in sedation. Pre-treatment with iproniazid accentuated the sedative effects. BOL anatgonized the aggravated sedation produced by concomitant administration of iproniazid and serotonin. The implications of these interactions are discussed. An effort has been made to distinguish between physiological and pharmacological effects of these drugs.

Claims that free serotonin, released by reserpine is a causative factor in parasympathetic manifestations of the latter drug are questioned. A hypothesis is advanced involving serotonin in the regulation of central autonomic function.

2. Acute treatment either with reserpine alone or BOL and reserpine induced the hypertrophy of the adrenals. Iproniazid prevented this hypertrophy. BOL did not modify further this action of iproniazid. However, chronic reserpinization was not evidenced by significant changes in adrenals.

Acute and chronic treatment with serotonin of animals, pretreated with iproniazid produced adrenal hypertrophy which was antagonized by BOL. Serotonin alone produced no significant changes. Thus, the effects of reserpine but not of serotonin were reduced by iproniazid whereas, the effects of serotonin and not those of reserpine were blocked by BOL.

In contrast with the response to the concomitant treatment with iproniazid and BOL and serotonin, the rats receiving the combined treatment of iproniazid, BOL and 5- HTP showed adrenal hypertrophy. However, the animals receiving only iproniazid and 5-HTP did not survive more than 6 hours of treatment.

In discussing the implications of these results an inhibitory role of serotonin in the regulation of ACTH secretion has been postulated.

3. Acute treatment with reserpine alone or BOL and reserpine did not produce significant changes in the accumulation of iodine by the thyroid. It, however, induced a complete block of daily thyroid secretion rate (TSR) despite about four and one-half times the control uptake which continued for 48 hours or more. Iproniazid again antagonized these effects. BOL had no effect on the ability of iproniazid to antagonize reserpine effects.

The total iodine content and TSR of rats undergoing chronic reserpinization was not determined. However, the zero hour and 24 hour radioiodine uptake and output constant were not found to be significantly different from the controls. Reduction in TSH secretion is envisaged. The reciprocal innervation of hypothalamic centers controlling ACTH and TSH secretion is hypothesized.

4. Acute treatment either with reserpine alone or with BOL and reserpine caused a fall in energy metabolism and a loss in body weight. Chronic reserpinization also affected the parameters mentioned during the first half of the treatment. This was followed by a recovery by

the 40th day. However, energy metabolism did not show a return to normal values. Other treatments did not elicit as marked or conclusive reductions in energy metabolism and body weight as reserpine did.

Pretreatment with iproniazid produced a marked reversal of reserpine effects that were not modified by BOL. It has been discussed that these effects are either a consequence of the initial "stress" produced by reserpine or due to peripheral pharmacological actions of the drugs used.

It is difficult to establish a neurohumoral transmitter role to serotonin and norepinephrine just on the basis of these results. An attempt has been made to correlate the findings of this study and those of other investigators engaged in the related fields.

The importance of neurohumoral transmitters in the brain is reemphasized. Of such transmitters serotonin and norepinephrine seem to be most important. It is still not clear which of the changes produced by reserpine can be directly related to brain amine concentration. Both peripheral and central action seems to be displayed by serotonin and reserpine and it is important to distinguish between physiological and pharmacological effects of serotonin and reserpine.

A peripheral site of action of reserpine that operates by blocking the peripheral adrenergic nerve mechanism by depleting catechol amines and a central site that acts by depleting the central serotonin stores is probable. The probability that MAO inhibitor iproniazid, prevents the release of amines can not be excluded.

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