

STUDIES IN ALLERGY

II. Serum Serotonin Levels in Nonallergic, Pretreatment, and Posttreatment Allergic Human Beings and in Normal and Sensitized Guinea Pigs

R. A. MacHaffie, M.D., Lucille R. Menebrocker, B.S.,** Delmar J. Mahler, Ph.D.,** and Anthony J. Barak, Ph.D.,*** Omaha, Neb.*

CONSIDERABLE controversy exists in the present-day biochemical and allergic literature concerning the role of serotonin (5-hydroxytryptamine) in allergic shock reaction and anaphylaxis. The presence of high serotonin levels in the lungs of the mouse, rat, and rabbit has been correlated with antihistamine-resistant anaphylactic shock in these animals.¹ The demonstration of increasing serotonin sensitivity following in vivo anaphylactic shock in the sensitized virgin guinea pig uterus² suggested that serotonin may play a role in some allergic and anaphylactic manifestations and that a clue might be found in the serum serotonin levels of patients with allergic sensitivity.

The present investigation was set up to study the serum serotonin levels of normal and sensitized guinea pigs. The serum serotonin levels of a group of normal nonallergic patients, as well as a group of allergic patients who entered the Allergy Inpatient and Outpatient Services of the Omaha Veterans Administration Hospital, were studied before and after treatment with water-soluble antigens.

METHOD

Serum serotonin levels were determined spectrofluorometrically. The method⁹ used was a modification of the procedure described by Waalkes and associates.³ In the present study, 2.5 ml. of double-distilled water (having minimal fluorescence) was added to 1 ml. of serum. Then 1 ml. of 10 per cent zinc sulfate followed by 0.5 ml. 1 N sodium hydroxide was added to deproteinize the serum. After centrifugation, 1 ml. of the clear supernatant was combined with 2 ml. of 9 N sulfuric acid. The fluorescence of the acidified sample was then determined in a Farrand spectrofluorometer (activation, 300 m μ ; fluorescence, 550 m μ). By addition of 0.002 micromoles of serotonin to a duplicate serum sample, we obtained an internal sample which was used for purposes of calculation.

From the Medical Service and the Medical Research Laboratory, Veterans Administration Hospital, Omaha, Nebraska.

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*Department of Internal Medicine, Omaha Veterans Administration Hospital; Instructor in Medicine, University of Nebraska and Creighton School of Medicine.

**Medical Research Laboratory, Omaha Veterans Administration Hospital.

***Medical Research Laboratory, Omaha Veterans Administration Hospital; Assistant Professor of Biochemistry, University of Nebraska School of Medicine.

The Animal Data.—The initial serum serotonin blood levels were obtained on forty-six female guinea pigs, each weighing 300 grams. Subsequently, eighteen of these animals were injected subcutaneously with alum-precipitated antigens, egg albumin, feathers, ragweed, and house dust. The serum serotonin levels of these animals were then studied monthly until the animals were sacrificed by intracardiac injection of antigen and the uterus was tested with water-soluble antigen in the Schultz-Dale apparatus. These animals were killed by a blow on the head, and the uteri were tested in the Schultz-Dale apparatus against water-soluble antigen.

The experimental group contained those animals that were anaphylactically sensitive to antigens (egg albumin, feathers, house dust, or ragweed) as evidenced by gross anaphylactic death and by contraction in the Schultz-Dale bath containing antigen.

The Human Data.—Forty-nine normal male medical students, physicians, laboratory technicians, and other hospital employees voluntarily supplied blood for normal serotonin levels. These persons had no allergic histories and were under no undue stress at the time the blood was taken.

Thirty-one allergic patients who suffered from a variety of allergic manifestations, including vernal conjunctivitis, allergic rhinitis, and seasonal bronchial asthma, were studied for serum serotonin levels. Thirty of these were placed under therapy with appropriate aqueous extracts of antigens, dust, ragweed, hemp, red top and, in several cases, tobacco and studied for serotonin serum levels after at least eight weeks of therapy with antigen doses adjusted to individual tolerance.

RESULTS

The mean serum serotonin level of the female guinea pigs in the normal group was found to be 0.285 mcg. $\pm$ 0.110 mcg. per cubic centimeter (Table I).

TABLE I. SERUM SEROTONIN LEVELS IN NORMAL GUINEA PIGS AND GUINEA PIGS SENSITIZED TO ANTIGENS

	(A) NORMAL GUINEA PIGS	(B) SENSITIZED GUINEA PIGS
Number	46	13
Mean serotonin level	0.285 mcg./c.c.	1. Initial 0.249 mcg./c.c. 2. Postsensitized 0.254 mcg./c.c.
Range	0.155-0.704	1. 0.176-0.303 2. 0.134-0.475
Standard deviation	0.110	1. 0.085 2. 0.107
Relationship of "t" test and significance	A to B ₁ A to B ₂ B ₁ to B ₂	1.09 P >0.05% 0.886 P >0.05% 0.132 P >0.05%

This is higher than that seen in the human subjects. Thirteen guinea pigs which were anaphylactically sensitized showed no statistically significant difference in serum serotonin level by "t" test, as noted in Table I, when tested initially and retested at a two-month interval after sensitization. The mean serotonin levels for the serum of this group of guinea pigs were 0.249 ± 0.085 mcg. per cubic centimeter before sensitization and 0.254 ± 0.107 mcg. per cubic centimeter after sensitization.

The results of the human serotonin levels are shown in Table II. The normal level for serum serotonin is 0.1527 ± 0.0668 mcg. per cubic centimeter and, when converted by hematocrit values for whole blood, agrees with levels found in the literature⁸ for the normal control subject.

TABLE II. SERUM SEROTONIN LEVELS IN NORMAL PERSONS, ALLERGIC PATIENTS, AND POST-TREATMENT ALLERGIES

	(A) NORMAL PERSONS	(B) PRETREATMENT ALLERGIC PATIENTS	(C) POSTTREATMENT ALLERGIC PATIENTS
Number	49	31	30
Mean serotonin serum level range	0.1527 mcg./c.c. 0.075-0.227	0.2047 mcg./c.c. 0.065-0.932	0.1972 mcg./c.c. 0.081-0.458
Standard deviation	0.0668	0.1566	0.0789
Relationship of "t" test and significance	A to B B to C A to C	2.05 $P < 0.05$	0.235 > 0.05 2.696 < 0.05

The serum serotonin level in pretreatment allergic persons is 0.2047 ± 0.1566 mcg. per cubic centimeter. This was considerably higher than the level found in normal persons. Statistical analysis for significance, by use of the "t" test, demonstrates the significance of this difference to be greater than at the 0.05 level.

Following allergic hyposensitization therapy with aqueous antigen extracts, the serum serotonin level remains high, the mean for this group being 0.1972 ± 0.0789 mcg. per cubic centimeter. The statistical difference between the mean of this group and that of the pretreatment allergic group is below the level of 0.05 significance, but it is above the 0.05 level of significance when compared with normal nonallergic human beings.

DISCUSSION

The role of 5-hydroxytryptamine (serotonin) in the body is complex because of its manifold properties and functions. The compound does not exist in whole blood or plasma, but it is found in serum as the result of platelet disintegration and in the brain, lungs, and small bowel. Humphrey and Jacques⁴ demonstrated its presence in rabbit serum in high concentrations following anaphylactic shock and suggested that the platelet's rapid disintegration during anaphylaxis was the etiological factor and the source of serotonin. Comroe and his associates⁵ studied the reflex and direct cardiopulmonary effects of serotonin in cats and found that minute doses of the order of 0.5 mcg. per kilogram of

body weight produced profound reflex cardiopulmonary changes, including bradycardia, hypopnea, and apnea, with bronchoconstriction and pulmonary vasoconstriction. These effects are mediated by serotonin-sensitive afferent fibers of the vagus nerve which are supplied by blood from the pulmonary circulation and ascending aorta.⁵ Herxheimer,⁶ working with aerosolized serotonin in the tracheobroncheal tree of the guinea pig, was able to produce a shock syndrome in the guinea pig similar to that caused by anaphylaxis, histamine, and acetylcholine. Guinea pigs sensitized to egg albumin and then desensitized by multiple antigenic exposure developed increasing tolerance to serotonin. Weissbach and co-workers¹ showed the presence of significant concentrations of serotonin of the order of 2.0 meg. per gram in the lung tissue of animals sensitive to serotonin, such as the mouse, rat, and rabbit, and much smaller amounts in the guinea pig which is (0.2 meg. per gram) a relatively nonsensitive species. It is interesting to note that the levels of monoamine oxidase (an enzyme thought to be a catalytic oxidizer of serotonin) were significantly higher in the guinea pig.³ These authors suggest that in those species in which serotonin is found in the lungs in significant quantities both histamine and serotonin should be considered as synergistic factors in allergic and anaphylactic reactions.

The higher level of serotonin which we have noted in the sera of persons with allergic sensitivity may be a response to allergic stress induced by contact of a sensitive person with an antigen or multiple antigen. Perhaps the platelets are sensitized as are the rest of the body tissues and respond by releasing excess serotonin, as suggested by Humphrey and Jaques.⁴

Fink and Gardner⁷ failed to demonstrate inhibition of anaphylaxis in guinea pig uteri and ileum in the Schultz-Dale apparatus by reserpine, yohimbine, and lysergic acid, and they postulate that serotonin plays no role in *in vitro* anaphylaxis. We agree with this concept as it applies to the guinea pig uterus in the Schultz-Dale bath. Our studies² and those of Fink and Gardner⁷ have shown that the guinea pig uterus and ileum are poor test organs for serotonin because of the low levels of positive responses (31 per cent of normal uteri and 40 per cent of sensitized uteri) in our series. We have also noted that a statistically significant increase in serotonin sensitivity occurs in this organ with *in vivo* anaphylaxis. We have postulated that the antigen-antibody—cellular membrane reaction in some unknown way sensitizes the cell to respond maximally to a serotonin level which formerly produced little or no response.²

Hyposensitization therapy appears to have no effect upon serotonin levels of the serum of posttreatment allergic patients. This was to be expected, since repeated exposure to antigen would tend to cause repeated stimulus for releasing of serotonin. In guinea pigs, however, there is no change in serotonin blood level as sensitization proceeds. This again demonstrates another essential difference between human atopy and anaphylaxis in animals.

SUMMARY

The sera of normal guinea pigs, sensitized guinea pigs, normal human beings, pretreatment allergic human beings, and posttreatment allergic human

beings have been examined for serotonin levels. Significant differences exist in allergic human beings in the direction of increased serotonin levels. These differences are not changed by allergic hyposensitization with appropriate antigens. In the guinea pig serum levels of serotonin do not change with sensitization. The meaning of these findings is discussed with reference to recent work on the role of serotonin, histamine, and antigen-antibody complexes in allergy and anaphylaxis.

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