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Effect of Administration of Amino Acids on Circulating Eosinophils and Lymphocytes in Normal and Adrenalectomized Rats. (19724)

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It has been known for some time that glutamic acid is the only amino acid that increases the oxygen consumption when added to nervous tissue *in vitro*(1,2). Weil-Malherbe(3) has made the claim that the therapeutic effect of the administration of glutamic acid in certain diseases of the central nervous system as reported by some investigators(4) was due to the liberation of adrenalin and not to the unique behavior of this amino acid on nervous tissue. (The controversy regarding the therapeutic efficacy of glutamic acid is outside the scope of this paper.) Since it has been shown that adrenalin will produce an eosinopenia and lymphopenia in animal and man(5-8), it was thought that similar effects should be produced by the administration of glutamic acid if the claim of Weil-Malherbe were correct.

A study was made of the effect of the oral administration of various amino acids and water or 0.5% saline on the blood picture of a group of white rats before and after adrenalectomy.

Procedure and methods. Each of a group of 12 male rats weighing approximately 200 g at start of the experiment was given 5 ml of a 5% solution of l-glutamic and l-aspartic acid as their mono-sodium salts, glycine, dl-α alanine and water by stomach tube with a rest period of several days between the administration of each of these constituents. The rats were not fasted overnight. The food was removed from the cages at 9 a.m. before the experiments were started. Water was allowed *ad lib.* The tail of the rat was warmed in water and blood was obtained by cutting the lateral vein of the tail near its distal end with a razor blade. Blood counts were made immediately before and 4 hours after the administration of the test material. A complete series with the various substances was carried out on each rat and the blood eosinophil count was determined by the method of Randolph (9). The complete series was then repeated on the same rats and total white, differential, and total lymphocyte counts were made. After completion of the 2 series the same rats were adrenalectomized and the same tests were repeated. Thus each rat was subjected to 20 experimental tests. Three of the 12 rats were lost as a result of the operation.

The adrenalectomized rats were given 5% glucose in physiological saline solution for drinking and the amino acids were dissolved in 0.5% saline instead of water. The rats were treated, otherwise, in the same manner as before adrenalectomy except that during the

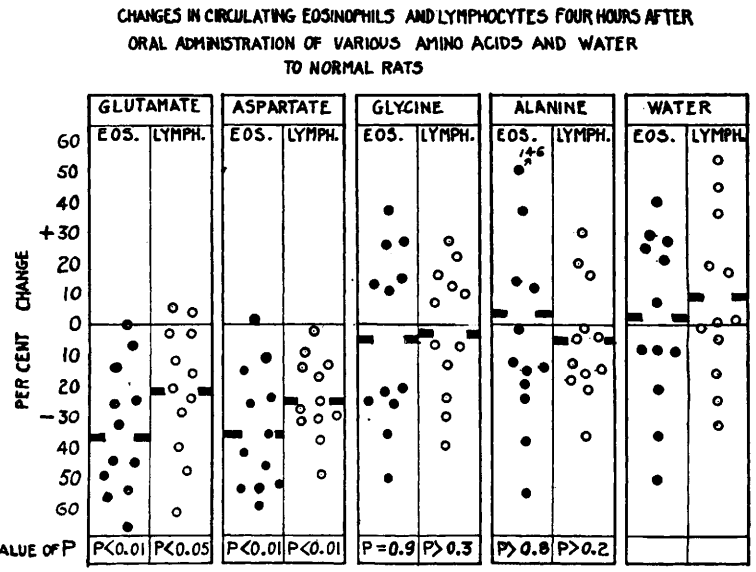

FIG. 1. Each point represents result of a test on each of 12 rats. Black dots represent % change in circulating eosinophils from the initial level, and open circles that of % change in level of circulating lymphocytes. Heavy horizontal bars mark the mean values. P values greater than .05 were not considered statistically significant.

4-hour experimental period the drinking water was replaced by 0.5% saline. The 9 adrenalectomized rats appeared well throughout the period of the experiments. In only one animal a very small adrenal rest was found on autopsy.

The effect of the administration of each amino acid on the per cent change in the absolute number of circulating eosinophils and lymphocytes was compared with that of water or saline as control for each rat. The data were analyzed statistically for significance.

Observations and results. Changes in the level of blood eosinophils. The administration of glutamic and aspartic acid resulted in a depression of the absolute eosinophil count in all of the normal animals but one in each group. The fall ranged from 7% to 67% in the former and from 11% to 60% in the latter with a mean of 36% and 35%, respectively. The per cent change in the absolute eosinophil count was compared for each rat against its water control and the statistical significance of the difference gave a value of $P < 0.01$ and $P < 0.05$, respectively. The administration of water, glycine and alanine gave marked plus and minus changes in the absolute eosinophil count (Fig. 1) and the changes due to these amino acids were not statistically significant.

Following adrenalectomy the administration of glutamic and aspartic acid was followed by a fall in the absolute eosinophil count except for one animal in each group which showed no change and one animal in the latter group which had a rise of 5%. The mean of the per cent change was minus 30.1% and minus 18%, respectively. These means were somewhat smaller than those obtained prior to adrenalectomy. The statistical significance of the changes compared against saline gave $P < 0.01$ (Fig. 2). The administration of 0.5% saline, glycine and alanine to the adrenalectomized rats again showed marked plus and minus changes in the eosinophil count and the effects of these amino acids were not statistically significant (Fig. 2). Thus the administration only of glutamic and aspartic acid produced a statistically significant change in the eosinophil count both before and after adrenalectomy although it was somewhat greater prior to adrenalectomy especially in the case of aspartic acid.

Changes in the blood level of lymphocytes. The administration of glutamic and aspartic acid was accompanied by a depression of the

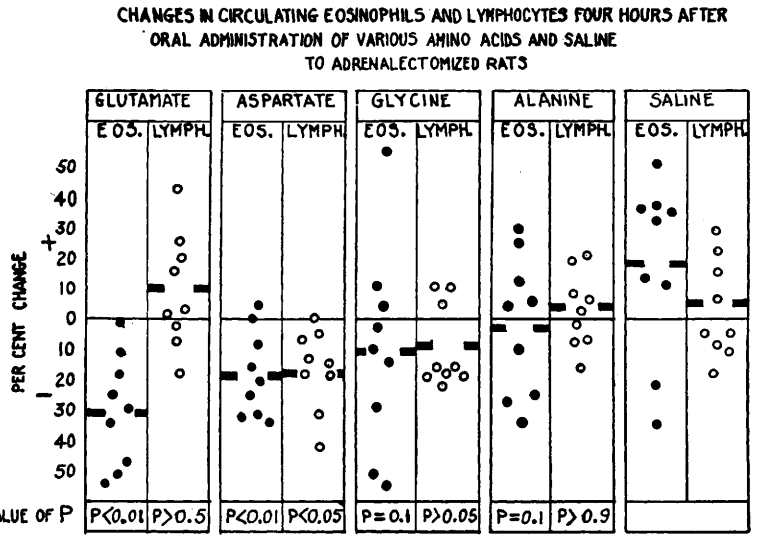

FIG. 2. Legend is the same as in Fig. 1 except that only 9 animals remained alive after adrenalectomy.

absolute lymphocyte count in all of the normal animals except in 2 of the former in which there was a slight rise. The mean of the per cent change was minus 20.7% and 23.8%, respectively. The per cent change for each rat compared with its water control was statistically significant with $P < 0.05$ and $P < 0.01$, respectively, for glutamic and aspartic acid.

The administration of water, glycine and alanine was accompanied by marked plus and minus changes in the absolute lymphocyte count (Fig. 1) which were not statistically significant.

Following adrenalectomy the administration of aspartic acid was accompanied by a depression of the lymphocytes in all the animals with a mean value of the per cent change equal to 17.7% as compared to 23.8% before adrenalectomy. The P value calculated as above against saline was $P < 0.05$ (statistically significant). Glutamic acid administration, however, was followed by marked plus and minus changes in the lymphocytes with a mean value of the per cent change equal to plus 0.9% and was not significant. This was in marked contrast to the effect on the eosinophils. The changes following glycine and alanine were not statistically significant (Fig. 2).

Discussion. The finding that the oral administration of sodium glutamate to normal rats produces a statistically significant depression of the blood eosinophil level, as compared to the effect of water, would be in harmony with the view of Weil-Malherbe(2) that glutamic acid stimulates adrenalin secretion. However, since a similar effect is produced in the same animals after adrenalectomy, it is obvious that some mechanism, other than adrenalin secretion and stimulation of the pituitary-adrenocortical system, accounts in large part for the observed effect. Were one to use the change in the lymphocyte count as an indicator, the absence of a statistically significant fall in the lymphocytes in the adrenalectomized rats would lend support to the view of Weil-Malherbe. (Studies on adrenal demedullated rats are being planned for further elucidation). However, since the administration of aspartic acid produced a statistically significant depression in the level of both circulating eosinophils and lymphocytes in the normal and adrenalectomized state, it is apparent that other mechanisms, besides adrenalin secretion and stimulation of the pituitary adrenocortical system, must play a role in affecting the level of these blood constituents. It is possible, however, that the lesser effects produced by glutamic and aspartic acid in the adrenalectomized animals indicate that part of their effects are produced through the

adrenals and perhaps via adrenalin secretion. It is hoped that further studies on adrenal demedullated rats may throw some light on this point.

The mechanisms which account for the difference between the mono and dicarboxylic amino acids and between glutamic and aspartic acid are obscure at present. It may be of interest to note that Awapara(10) has reported that the adrenals appear to play some significant role in the metabolism of the dicarboxylic amino acids.

Summary and conclusions. 1. A study was made to determine whether the oral administration of glutamic acid produced an increased secretion of adrenalin by employing the changes in the blood eosinophil and lymphocyte level as indicators. 2. Glutamic acid, aspartic acid, glycine, alanine, and water or saline were administered to a group of white rats before and after adrenalectomy and the effects on the levels of the blood eosinophils and lymphocytes were determined. 3. Only the dicarboxylic amino acids produced a statistically significant depression of the eosinophils in the normal and adrenalectomized animals. However, in the latter animals the lymphocytes also were depressed by aspartic acid but not by glutamic acid. 4. The results obtained in adrenalectomized rats showed that the levels of the blood eosinophils and lymphocytes could be influenced by other mechanisms than the pituitary-adrenal system. 5. The possible significance of the small difference observed between normal and adrenalectomized rats regarding eosinophil changes is discussed.

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Effects of Adrenal Ligation and Adrenergic Blockade on Pressor Responses to Acetaldehyde in Dogs.* (19725)

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The use of tetraethylthiuram disulfide (Antabuse) in the treatment of chronic alcoholism has stimulated interest in the mechanisms involved in the Antabuse-alcohol reaction. At the present time, it is believed that the acetaldehyde formed from ingested alcohol produces the symptoms of the Antabuse-alcohol reaction in which hypotension is characteristic(1). Nelson(2) has shown that acetaldehyde causes a transient pressor response in dogs after both carotid arteries are clamped, the vagus nerves sectioned, the adrenal vessels ligated, and after ganglionic blockade induced by nicotine. Reversal of the pressor response by the adrenergic blocking drug 2-(N, p-tolyl-N [m′ oxyphenyl] aminomethyl) imidazoline (C-7337) has been demonstrated by Christensen(3) to occur in the intact anesthetized dog.

These observations indicate that acetaldehyde is a peripherally acting drug. The present experiment was undertaken to confirm 2 aspects of the evidence: 1) that the pressor response is not due to the release of epinephrine from the adrenal medulla; 2) that the pressor response is reversed by an adrenergic

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