allowed before beginning the assay; during this time the muscle relaxed maximally. Strips cut from crops which had contained food when the chicks were killed, usually exhibited powerful and erratic spontaneous movements and were unsuitable for assay preparations. Some strips of crop taken from starved chicks also exhibited spontaneous movements but these were rarely troublesome since they were regular and small in amplitude.

The standard solutions and the solution to be analysed were first diluted with Krebs solution so that the required doses were contained in volumes of 1 ml. Doses were added by emptying the bath from the bottom and refilling from the top with 1 ml of the required solution delivered from a pipette. The drug was left in contact with the tissue for 60 sec and then washed out by refilling the bath twice with fresh Krebs solution. Doses were added in the form of a Latin square design at constant intervals of 6 to 9 min. The preparations remained stable throughout at least 2 complete Latin squares (32 doses) and many were sensitive to as little as 10^{-12} g (1 picogram) of 5-HT. At room temperature the weak solutions of 5-HT remained stable throughout the assay. Raising the temperature of the Krebs solution to 32° slightly increased the sensitivity of the assay. However, it was then necessary to maintain the 5-HT solutions at the same temperature and this caused progressive decomposition. In 7 assays, the results of which were analysed statistically, the indices of precision (λ) were less than 0·05 and the fiducial limits of the potency ratios all fell between 88 and 112%.

The preparation was also found to be sensitive to histamine (about 1 ng/ml) and acetylcholine (about 10 ng/ml in the absence of anticholinesterase) and preliminary experiments suggest that it may be used to assay these substances also. Each agonist was selectively blocked by an appropriate antagonist so that any one might be assayed in the presence of the other two. Histamine was completely blocked by mepyramine (10 ng/ml), 5-HT by bromolysergic acid diethylamide (1-5 μg/ml) and acetylcholine by atropine (10 ng/ml). This concentration of atropine approximately halved the sensitivity to 5-HT but even in the presence of atropine the preparation was considerably more sensitive than other available preparations. Because of the high sensitivity, body fluids to be assayed must be extensively diluted with Krebs solution and it is hoped that interfering substances may thereby be inactivated. Assays made on samples obtained from 6 normal adults indicate that the method is suitable for the estimation of free 5-HT in urine.

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Effects of ammonium chloride and sodium bicarbonate on resistine levels in rats.

SIR,—Different tissue-damaging procedures result in enhanced production of "resistine" (Karady & Kovacs, 1948), a substance which exerts an antihistamine action and which reduces histamine release (Kardy, Gecse & Horpacsy, 1962). Since acidifying or alkalizing treatments often produce favourable results in clinical practice (mainly in allergic disease), it was of interest, in order to shed some light on the mechanism of the favourable effect of these treatments, to follow the changes in resistine levels in rats after treatment with acidifying and

alkalizing compounds. Resistine levels were measured by determining the ability of rat blood to protect guinea-pigs from convulsions produced by histamine (Prokai, Mustardy & Karady, 1961; Karady, Prokai & Mustardy, 1961). At the same time the ability of the blood to protect guinea-pig convulsions produced by 5-hydroxytryptamine (5-HT) and acetylcholine aerosols was tested to determine the specificity of resistine.

Groups of 14 or more rats received either ammonium chloride (680 mg/kg) or sodium bicarbonate (600 mg/kg) by stomach tube daily for 9 days. Twenty-four hr after the last dose, they were killed and the sera of rats belonging to the same group were mixed and injected intraperitoneally into groups of guinea-pigs which had been previously tested for sensitivity to aerosols of histamine (0·15%), 5-HT (0·2%) and acetylcholine (0·6%). Six hr later, the sensitivity to the three agents was re-tested. In each case, the time to the first convulsive cough was taken as the end-point, and the results are calculated as mean percentages of

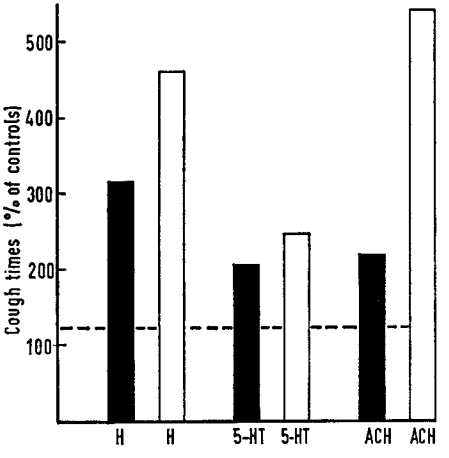

FIG. 1. Effect of sera of rats treated with ammonium chloride (■) or sodium bicarbonate (□) on the cough times of guinea-pigs subjected to histamine, 5-HT and acetylcholine aerosols. Values greater than the broken line are highly significant ($P<0.01$). Note that both treatments prolong the cough times in each case.

cough-times of guinea-pigs before treatment. The results recorded in Fig. 1 show that both treatments stimulated rats to produce resistine, as indicated by the significant increases in the histamine aerosol times. However, the effect is not specific as cough times to 5-HT and acetylcholine were also increased. This suggests that resistine is antihistamine, anti-5-HT and atropine-like in nature.

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