None of the six above-mentioned compounds, on intravenous administration, antagonised tremorine-induced tremors.

For optimum activity in producing loss of righting reflex and anti-leptazol activity, the following requirements appear to be the necessary minimum—(*a*) a pregnane nucleus, (*b*) a 2β-morpholino-group, (*c*) a 3α-hydroxyl group.

Although the amino-esters of 21-hydroxypregnanedione of Figdor & his colleagues (1957) had general anaesthetic activity and the compounds (I–VI) we have investigated possessed loss-of-righting-reflex and anti-leptazol activities, there is agreement on two points: (*a*) the morpholino-substituted steroid was the most active and (*b*) nuclear substitution decreased potency.

A further compound, (VII), 3β-acetoxy-5α-hydroxy-6β-morpholino-5α-pregnan-20-one, produced convulsions in mice and these superficially resembled leptazol convulsions.

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A sensitive preparation for the assay of 5-hydroxytryptamine

SIR,—The crop of the young chick contracts strongly in response to minute amounts of 5-hydroxytryptamine (5-HT) and has been used as a sensitive assay preparation.

Chicks (Silver Link) up to 7 days old were starved overnight to empty the crop and then killed with ether. The crop was removed and placed in a petri-dish containing Krebs-Henseleit solution (g/litre: NaCl 6·95, KCl 0·34, $CaCl_2$ 0·28, KH_2PO_4 0·162, $MgSO_4$ 0·294, $NaHCO_3$ 2·1 and dextrose 2) at room temperature. It was opened by a longitudinal cut in the wall of the attached portion of oesophagus and a strip of tissue about 3 mm wide was then cut transversely from the middle of the opened crop. The strip was suspended in a Perspex bath of just over 1 ml capacity containing Krebs solution at room temperature (20-23°) which was bubbled slowly with 5% carbon dioxide in oxygen. One end of the strip of crop was attached to an isotonic frontal writing lever loaded with 4 g and magnifying the contractions 12 times. An interval of 30-60 min was

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