distributed variables. Such ratios should be used with caution. Furthermore, the derivation of systemic blood clearance of drugs should be from the measured blood drug for maximum confidence in the value.

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# Effect of TRH on acid secretion in the mouse stomach

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Evidence of TRH (thyrotropin-releasing hormone) localization in the entire gastrointestinal tract (Morley 1979) has prompted investigations on the effects of this peptide. Several studies to elucidate the role of TRH in secretory and motility mechanisms have been made (Tonoue et al 1979; Dolva et al 1982; Soldani et al 1983; Oouchi & Ichikawa 1985). However, the results concerning the involvement of TRH in gastric acid secretion are somewhat contradictory, because the type of effect, stimulatory or inhibitory, appears to be related to the route of TRH administration (intravenous versus intracerebroventricular) (Taché et al 1980) and to the secretory stimulus employed (locally or centrally acting). A consistent feature of TRH appears to be its inhibitory effect when gastric acid secretion is elicited by a centrally mediated stimulus, e.g. food, sham feeding, insulin (Gascoigne et al 1980; Konturek et al 1981). As studies on the TRH peripheral effect have all been performed in-vivo, it seemed worthwhile to use a model devoid of extrinsic regulatory influences, therefore we chose the isolated stomach of the mouse, as described by Angus & Black (1978).

Compared with other isolated preparations (oxyntic cells, glands or mucosal sheets), this model has two advantages: preservation of the cellular architecture, necessary for responses to physiological agents, and maintainance of the intrinsic innervation. Therefore the mouse isolated stomach may be considered to be the basic physiological unit for acid secretion (Black & Shankley 1985). This preparation has been widely used in studies on secretion induced by histamine $H_2$ and cholinergic agents (Angus & Black 1979, 1982; Szelenyi & Postius 1984).

Our results showed that TRH (Relefact TRH, obtained from Hoechst, Italy) in the range of 1–30 μM had no effect on basal gastric acid secretion. However, when secretion was evoked by vagal excitation (electrical stimulation of oesophageal stump at 20 Hz, 10 V, 1 ms, following 15 min incubation with TRH), the peptide induced a significant ($P < 0{\cdot}05$) inhibition of secretion. This effect of TRH was dose-dependent in the concentration range of 3·2–16·5 μM, the 50% inhibiting concentration of TRH, IC50, being 6·26 μM. TRH affected the secretory peak response, reducing the maximal rate of acid output from 250 to 100 nM $H^+$ $min^{-1}$, without interfering with the time of onset (about 10 min). Unlike other antagonists of vagal stimulation, such as atropine and clonidine, which are able to cause a full blockade, the maximal inhibition induced by TRH (9·4 μM) was only about 70%. Neither increasing the concentration of TRH nor adding of the enzymatic inhibitor, phenylmethyl-sulphonylfluoride, could produce a greater antisecretory response. Vagal stimulation of acid secretion is known to depend on release of both acetylcholine and histamine, as it is sensitive to muscarinic (atropine) or $H_2$ (metiamide) blockers (Angus & Black 1982). TRH may act via a different mechanism, since it was completely ineffective when acid secretion was elicited by the muscarinic agonist bethanechol or by histamine.

It is a matter of speculation at which step of the vagal pathway TRH might interfere. Recent studies show that besides acetylcholine, vagal stimulation induces a release of other neurotransmitters, such as bombesin (Nishi et al 1985), VIP (Larsson et al 1976) and perhaps other peptides. These in turn control gastric acid secretion by releasing other substances, e.g. gastrin, somatostatin (Hirschowitz 1982). TRH might interact with one of these peptides to modify the gastric acid response.

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This hypothesis appears to be consistent with the finding that TRH inhibition is often incomplete and also with the in-vivo observations describing a conspicuous effect of TRH when acid secretion is stimulated via a neural, centrally mediated pathway.

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# Statistical analysis of gastrointestinal transit time of pharmaceutical formulations: comments on the letter by Devereux & Newton

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The letter by Devereux & Newton (1985) is a useful contribution since it highlights the problem of the statistical analysis of data from experiments where crossover and group comparisons are combined. Generally such a design should be avoided if possible but since data of this kind may be produced it is important to be able to perform an optimal statistical analysis.

We agree that it is incorrect simply to forget about the matched pairs and analyse the data by methods for comparing two independent samples. The method proposed by Devereux & Newton is known to be the optimal one, *but only if the standard deviations are known and the variables are normally distributed.* When the standard deviations are unknown but estimated with high precision, i.e. many degrees of freedom, the method would certainly still be satisfactory. However, in the actual case discussed, the standard deviation of the differences is estimated with only 2 degrees of freedom in the matched pairs case and this gives a very inaccurate estimate, a fact overlooked in developing the method proposed by Devereux & Newton.

The pitfall of ignoring the inaccuracy of the standard deviation estimates is probably as invalidating for the final result as that of ignoring the matched pairs!

Take as an example the figures for intestinal transit given by Christensen et al (1985). The three matched pairs give $t_1(2) = 7{\cdot}60$ which is statistically significant at the 5% level (two-sided test). The 2 + 5 unmatched pairs give $t_2(5) = -1{\cdot}08$ which is not statistically significant at the 5% level (two-sided test). The test statistic called SND proposed by Devereux & Newton is also a weighted average of these two *t*-values, thus

$$\mathrm{SND} = \frac{t_1\, s_{d2} + t_2\, s_{d1}}{\sqrt{s^2_{d1} + s^2_{d2}}}$$

where $s_{d1}$ and $s_{d2}$ are the estimated standard errors of $d_1$ and $d_2$ based on matched and unmatched pairs respectively.

Since $s_{d2} = 54{\cdot}4$ and $s_{d1} = 20{\cdot}5$, the $t_1$ value is given more than double weight as compared with the $t_2$ value, although $t_1$ has infinite variance whereas $t_2$ has a variance of 5/3 if $E(d_2) = 0$.

The variance of a non-central t variable with ν degrees of freedom and non-centrality parameter, δ is

$$V(t_\nu(\delta)) = \frac{\nu}{\nu - 2}(1 + \delta^2) - \left(\sqrt{(\nu/2)}\,\frac{\Gamma((\nu - 1)/2)}{\Gamma(\nu/2)}\,\delta\right)^2$$

see for example Johnson & Kotz (1970).

Another problem arises because the two estimates $\bar{d}_1 = 156{\cdot}0$ and $\bar{d}_2 = -58{\cdot}7$ seem to be statistically significant. If this is true it makes no sense to pool the two estimates at all.

Assessing the statistical significance of the difference between $\bar{d}_1$ and $\bar{d}_2$ is a comparison of means in two normal distributions, which is easily done if the standard

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