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A new method for the assay of orally active hypolipaemic agents

In 1943, Hahn reported that heparin was able to clarify hyperlipaemic sera while Anfinsen, Boyle & Brown (1952) showed that the plasma of animals previously treated with heparin contained a clearing factor which is responsible for the diminution of absorbance of lipaemic sera mixed with such plasma. Several aspects of the mechanism have been discussed by Robinson & French (1960). Although the mechanism of the clarifying activity of heparin has not been completely elucidated, Wolff & Brignon (1959) suggested that it releases cellular enzymes, whose coenzymes are lipoproteins. Other substances having an anti-lipaemic effect have been sought, as heparin is effective only parenterally and its anticoagulant activity is undesired (Oliver, 1967).

Several substances called heparinoids were proposed as orally active hypolipaemic agents by Bianchini & Osima (1959) and Szabó, Larrocha & Sandor (1968). These were obtained either from natural sources or by chemical synthesis. No simple method is known for a laboratory assay of their hypolipaemic activity using the oral route. We propose the following method for this purpose.

The method is based on the inhibition of the hyperlipaemic effect of ACTH (Meakin & Nelson, 1960; Robinson & French, 1960; Hollenberg, Raben & Astwood, 1961; Lebowitz, Breyant & Frohman, 1965; Oliver, 1967). We observed that several natural heparinoid preparations administered by stomach tube antagonized the ACTH-induced increase of free fatty acids in rabbits. Albino rabbits of 2·5–3 kg and of either sex, were used. The animals were allowed food freely. They were then placed in individual cages and blood was extracted from the marginal ear vein 30 min before the injection of ACTH.

Blood FFA were assayed by Dole's method (1956). Every rabbit received subcutaneously 60 units of ACTH. After 2 and 4 h, respectively, blood was extracted and assayed for FFA. Initial values of blood FFA varies between 0·30 and 1·51 μ equiv/ml with a mean value 0·74. Two h after giving 60 units of ACTH the values were between 3·70 and 8·90 μ equiv/ml, with a mean value 6·52. After 4 h there was a diminution of these values (between 0·70 and 7·40, mean value 3·98), without return to the original levels. An activity coefficient, R, was calculated from the following formula:

$$R = \frac{\text{FFA at 2 h} - \text{FFA at 0 h}}{\text{FFA at 0 h}}$$

The coefficient of the control group treated with ACTH alone was 7·8.

Another group of rabbits was treated by stomach tube by a natural heparinoid, simultaneously with the injection of ACTH. This heparinoid was obtained from bovine duodenum by alkaline extraction and subsequent precipitation with acids and organic solvents according to Fischer, Szabó & Stark (1965). Doses between 10–100 mg/kg were used. A control group was treated similarly. The results are shown in Table 1. The differences between the concentrations (C) at 0 and 2 h for each animal were established and the mean values of these differences for each group studied were calculated.

The analysis of the variance shows that the difference between the control group (5·78) and the mean value of the treated groups (3·24) is very significant ($F_{1\cdot36} = 33, 9$; $P < 0{\cdot}001$). There is also a highly significant difference between the five mean values of the groups treated with different doses ($F_{4\cdot36} = 6{\cdot}7$; $P < 0{\cdot}001$). The regression line between effects (C_2–C_0) and the log of the dose was estimated. The equation of the line is: $y = -3{\cdot}83 \times +9{\cdot}75$. The slope is highly significant ($F_{1\cdot36} = 17, 23$, $P < 0{\cdot}001$), and indicates clearly the relation between effect and log doses. The deviation from the line corresponds to $F_{3\cdot36} = 3{\cdot}25$; $P < 0{\cdot}05$.

Table 1. *Effect of heparinoid preparation on the serum FFA concentration* (μ equiv/ml) *after ACTH administration.*

Doses	0 h	2 h	4 h	Coefic.
Control group	0·60 ± 0·10	5·13 ± 0·28	—	R = 7·8
ACTH 60 U + 10 mg/kg Hep	0·37 ± 0·12	4·46 ± 0·24	—	R = 10·2
ACTH 60 U + 20 mg/kg Hep	1·16 ± 0·05	6·89 ± 0·27	4·78 ± 0·28	R = 4·94
ACTH 60 U + 40 mg/kg Hep	1·12 ± 0·09	5·38 ± 0·57	5·04 ± 0·72	R = 3·8
ACTH 60 U + 80 mg/kg Hep	1·72 ± 0·61	4·89 ± 0·29	4·05 ± 0·91	R = 1·84
ACTH 60 U + 100 mg/kg Hep	1·05 ± 0·13	2·31 ± 0·69	2·07 ± 0·49	R = 1·2

Table 1 shows that the activity coefficient varies according to the dose of heparinoid. A good dose-response curve was obtained. The amount producing 50% inhibition was regarded as a minimal effective dose. This corresponds to 40 mg/kg of the assayed heparinoid and is designated arbitrarily as 10 unit/mg. The inhibition of ACTH effect on plasma lipids by the heparinoid administered by stomach tube provides evidence for its enteric absorption.

We propose the method for standardizing orally active heparinoid preparations.

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Antagonism by *p*-chlorophenylalanine of late tranylcypromine toxicity

Overdoses of tranylcypromine lead to a toxic syndrome characterized clinically by hyperactivity, hyperpyrexia, seizures and other signs of marked central nervous system stimulation (Baldridge, Miller & others, 1962; Bacon, 1962; Jacobziner & Raybin, 1963; Atkinson & Ditman, 1965). At a later stage the syndrome might include psychotic behaviour (Baldridge & others, 1962). Bacon (1962) and Jacobziner & Raybin (1963) stated there is no specific antidote available; this continues to be the case. Nor is there a consensus regarding the mechanism of the toxic reactions. Thus though tranylcypromine is a very powerful and lasting monoamine oxidase (MAO) inhibitor (Pletscher, Goschke & others, 1961; Atkinson & Ditman, 1965), it has not proved possible to relate clinical changes to the effects of tranylcypromine on the metabolism of any one single amine. Moreover tranylcypromine also possesses amphetamine-like properties (Baldridge & others, 1962; Atkinson & Ditman, 1965) which tend to be most evident during an initial period after administration. Probably because of the latter, the pharmacological actions of tranylcypromine exhibit some biphasicity with respect to time. Such biphasicity has been observed by Ling (1962) in the response of the electroencephalogram (eeg) of the cat to intravenously administered tranylcypromine, an initial phase of transient and a secondary phase of intense activation being separated by a period of deactivation. We have observed a biphasicity with respect to the toxic actions of tranylcypromine in mice and have found *p*-chlorophenylalanine (*p*-CPA) to protect mice against late, but not early, tranylcypromine toxicity.

Male CWF mice (Carworth Inc., New City, N.Y.) 22–33 g, were divided into two groups, one group was administered 100 mg/kg *p*-CPA dissolved in 2% saline on three successive days, the second group 2% saline injections on the same schedule. Twenty-four h after the last injection the mice were isolated under individual 6″ diameter glass bowls inverted over a wire grid and injected in staggered fashion at 1·5 min intervals with tranylcypromine (Gessner, Soble unpublished findings).

Five doses of tranylcypromine were used, equal numbers of *p*-CPA and saline pretreated animals being injected any given dose. The animals were checked to determine whether they were still alive at 2, 4, 8, 16 and 32 h from the time of the tranylcypromine injection, death being defined as a silent ecg tracing. The cumulative results obtained are given in Table 1, expressed as a function of dose. When these results are expressed in terms of the mortality observed during given time period among the animals alive at the beginning of that period (Fig. 1), the biphasic nature of tranylcypromine toxicity becomes apparent. Moreover upon inspection of Fig. 1, it can be seen that while *p*-CPA pretreatment has no effect on early mortality following tranylcypromine administration, it does appear to have protected the mice from late tranylcypromine mortality. Statistical analysis indicates that the 32 h mortality among those animals surviving tranylcypromine administration by 4 or 8 h and the 16 h mortality among the