serious symptoms of adrenergic blockade after the administration, but recovered within 6 h. Blood was taken before and 24 h after injection and serum cholesterol was estimated by the SPERRY-WEBB method[9].

The serum cholesterol decreased slightly after administration of nor-adrenalin (mean: $-3.6\%$, $p < 0.02$), and the change of cholesterol by the serotonin treatment was not significant. However, 24 h after single i.v. injections of Dibenzyline the serum cholesterol increased and that to a highly significant degree (Table).

*Discussion.* Like a number of other adrenergic blocking agents, Dibenzyline produces its effect by reducing the pressor action of adrenalin. From numerous investigations which showed that adrenalin elevates the serum cholesterol level, its seemed reasonable to assume that any anti-adrenergic agent will lower the cholesterol level and indeed there are reports that serum cholesterol decreases after administration of some ganglion blocking drugs[7,8,10]. Contrarily, I have found that in short-term experiments on rabbits, an adrenergic blocking substance, namely Dibenzyline, has a cholesterol raising effect. There is only one investigation in which the influence of Dibenzyline on serum cholesterol was studied: Hollister reported that Dibenzyline lowers serum cholesterol, but the statement was based on tests in only 2 patients, one of which showed an equivocal decrease. Moreover, this author's tests were carried over several weeks, and as he himself indicated, not with sufficient dosage. For these reasons, the results are not comparable.

The explanation why Dibenzyline failed to decrease the level of serum cholesterol and even raised it, presents difficulties. According to recent reports[11,12] on the action of adrenalin, the mobilization of lipids from tissue to serum is controlled by adrenalin. However, according to data which we obtained, neither nor-adrenalin nor serotonin which have adrenergic action always raise serum cholesterol levels in rabbits.

Furthermore the weakness of the animals after injection of Dibenzyline has to be considered as having a possible effect on cholesterol metabolism and thus may counteract an hypothetical specific serum cholesterol lowering effect of Dibenzyline. This weakness may be considered an expression of stress which is said to have some effect on cholesterol metabolism[13,14]. However. this effect is very inconsistent in animals and humans. Nevertheless, such possibility of interference should not be overlooked. It therefore remains to be established whether Dibenzyline has a direct action on serum cholesterol preventing its catabolism, which is unlikely; whether the rise in cholesterol is due to vascular phenomena, or whether a metabolic disturbance is responsible.

5.0 ml of 0.5 mg per ml aqueous solution of Dibenzyline per kg body weight were administered intravenously

| Rabbit | Sex | Body weight g | Cholesterol in mg% before test | Cholesterol in mg% after test | % change |
|---|---|---|---|---|---|
| 1 | M | 4930 | 27 | 37 | + 37.0 |
| 2 | M | 2370 | 32 | 38 | + 18.7 |
| 3 | M | 3680 | 33 | 41 | + 24.2 |
| 4 | M | 2510 | 38 | 48 | + 26.3 |
| 5 | M | 2190 | 43 | 48 | + 11.6 |
| 6 | F | 2250 | 48 | 63 | + 31.3 |
| 7 | M | 3240 | 63 | 64 | + 1.6 |
| 8 | F | 2500 | 82 | 107 | + 30.4 |
| 9 | M | 3320 | 86 | 76 | − 11.6 |
| 10 | M | 2600 | 88 | 97 | + 10.2 |
| 11 | M | 3140 | 98 | 118 | + 20.4 |
| 12 | F | 2010 | 104 | 109 | + 4.8 |
| 13 | F | 2750 | 104 | 125 | + 20.2 |
| 14 | M | 3830 | 135 | 161 | + 19.2 |
| 15 | M | 2910 | 180 | 196 | + 8.9 |
| 16 | F | 2820 | 186 | 175 | − 5.9 |
| 17 | F | 4390 | 371 | 308 | − 17.0 |
| | | | | mean | + 13.5 |

$t = 3.6$, $p < 0.01$

No. 1: very weak, hypersalivation and enophthalmos after injection.

*Zusammenfassung.* Im Gegensatz zu den Beobachtungen, die zeigten, dass adrenergische Substanzen Serumlipoide einschliesslich Serumcholesterin erhöhen, wird in kurzfristigen Versuchen an Kaninchen eine etwas unregelmässige Senkung des Serumcholesterins nach Injektion von Noradrenalin und keine signifikante Beeinflussung des Serumcholesterins nach Injektion von Serotonin gefunden. Dibenzylin erhöhte in kurzfristigen Versuchen das Serumcholesterin ausgesprochen signifikant.

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## The Effect of pH on Enzymatic Formation and Inhibition of Norepinephrine Synthesis

The extraction and purification of an enzyme from adrenal medulla which catalyzes the conversion of dopamine to norepinephrine was recently described[1]. It was shown that this enzyme is non-specific and also catalyzes the conversion of other phenylethylamines and phenylpropylamines to the corresponding $\beta$-hydroxy compounds[2,3]. In view of these findings, the enzyme was named phenylamine-$\beta$-hydroxylase[3].

The present communication is concerned with the effect of pH upon the rate of conversion of dopamine to norepinephrine and epinine to epinephrine by phenylamine-$\beta$-hydroxylase. The effect of pH on the inhibition rate of dopamine to norepinephrine conversion by various inhibitors was also examined.

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The experiments were carried out as previously described[3]. The buffer used in each experiment is described in the respective Tables. Norepinephrine and epinephrine were assayed fluorometrically, and in some experiments norepinephrine was assayed by a modification of the periodate method[1,3]. The effects of pH and of the buffer composition on the norepinephrine formation from dopamine and epinephrine formation from epinine are presented in Table I. The optimum rate of formation for norepinephrine and epinephrine occurs at pH 5.5. The formation of norepinephrine and epinephrine is diminished in an incubation mixture with phosphate buffer as compared with a corresponding incubation mixture with acetate buffer. The inhibitory effect of phosphate buffer may be due to binding of some cations with phosphate. This conclusion is supported by our recent findings that ethylenediamine tetraacetate also inhibits the norepinephrine formation. Since both substrates, dopamine and epinine, remain in the cationic form over the entire range of the investigated pH, the effect of the pH is not due to ionization of the substrates' amino group. The effects of the pH may be due to ionization of enzymatically active sites or of co-factors such as ascorbic acid or fumarate. The pH may also affect the stability of the substrate enzyme or intermediate product enzyme complex. In the latter complex, the amino group must be protected during the oxydation at the $\beta$-carbon by its binding to the active enzyme site.

Tab. I. The effects of pH and buffer composition on the enzymatic formation of norepinephrine from dopamine and epinephrine from epinine

| pH | Formation of norepinephrine[a] in µg/ml | | Formation of epinephrine[b] in µg/ml | |
|---|---|---|---|---|
| | Ascorbate added | omitted | Ascorbate added | omitted |
| 6.5 | 135.0 (75.0)[c] | 35.0 | 67.0 (35.0)[c] | 31.0 |
| 6.0 | 170.0 | — | 82.0 | — |
| 5.5 | 230.0 | 19.0 | 94.0 | 27.0 |
| 5.0 | 164.0 | — | 78.0 | — |
| 4.5 | 60.0 | — | 27.0 | — |

[a] The concentration of the substrate was 2 µmoles of dopamine per ml.
[b] The concentration of the substrate was 4 µmoles of epinine per ml.
[c] These values were obtained from an inhibition mixture in which 0.5 ml of 0.2 *M* phosphate buffer was added, while to the other incubation mixtures 0.5 ml of 0.2 *M* acetate buffer was added.

Tab. II. Inhibition of norepinephrine synthesis by DOPA, tryptamine, and adrenalone as a function of pH

| pH | % inhibition Inhibitors[a] | | |
|---|---|---|---|
| | DOPA | Tryptamine | Adrenalone |
| 6.5 | 10.0 | 10.0 | 55.0 |
| 6.0 | 36.0 | 16.0 | 48.0 |
| 5.5 | 50.0 | 17.0 | 29.0 |
| 5.0 | 43.0 | 11.0 | 5.0 |
| 4.5 | 12.0 | 8.0 | 0.0 |

[a] The concentration of DOPA was 4 µmoles/ml, of tryptamine 4 µmoles/ml, and of adrenalone 0.2 µmoles/ml. The concentration of the substrate dopamine was 2 µmoles/ml in all incubation mixtures. All incubations were carried out in acetate buffer.

Whether the effect of pH is due to the ionization of ascorbic acid was investigated by incubation of dopamine and epinine with and without the addition of ascorbic acid. It is evident from Table I that the formation of norepinephrine from dopamine in an incubation mixture without ascorbic acid is lower at pH 5.5 than at pH 6.5. The decrease of epinephrine formation from epinine is not affected as much by the above changes in pH. These results may be explained by the findings of LEVIN et al.[4] that in the absence of ascorbic acid the hydroxylation of dopamine or epinine is dependent on the formation of dopamine quinone or epinine quinone. At a lower pH, such as 5.5, dopamine quinone is not as readily formed as epinine quinone and therefore the formation of norepinephrine from dopamine in the absence of ascorbic acid decreases to a greater extent with pH. This suggests that the hydroxylation of dopamine or epinine is enhanced by ascorbic acid as well as by the corresponding quinone formation. The extent to which the $\beta$-hydroxylation is enhanced by ascorbic acid or by quinone formation varies with pH. It is also evident that the $\beta$-hydroxylation of epinine is supported to a greater extent by the quinone formation than is the $\beta$-hydroxylation of dopamine.

Various compounds were studied as possible inhibitors of dopamine to norepinephrine conversion by phenylamine-$\beta$-hydroxylase. The inhibition rate of norepinephrine synthesis by DOPA, tryptamine, and adrenalone (3,4-dihydroxy-$\alpha$-methylaminoacetophenone) was measured as a function of pH. DOPA and tryptamine inhibition has its optimum at pH 5.5 while adrenalone inhibition has its optimum at pH 6.4 (Table II). At the present time it is not clear what factors are responsible for the effects of pH on the inhibition by DOPA or tryptamine. However, the optimum inhibition by adrenalone at pH 6.4 and the sharp decrease at lower pHs indicates that a nucleophilic group on the enzyme site is necessary for the formation of the enzyme adrenalone complex. The change of the molar extinction coefficient of adrenalone at pH 5 from $\Sigma_{max}$ 11000, $\lambda_{max}$ 230 to $\Sigma_{max}$ 8500, $\lambda_{max}$ 230 at pH 6 indicates that the carbonyl group at pH 6 is enolized to a greater extent, and that this form is responsible for the adrenalone enzyme complex.

Adrenalone was not only found to be a potent inhibitor *in vitro*, but in preliminary studies it was also shown that it inhibits norepinephrine formation *in vivo*[5,6].

*Zusammenfassung.* Die enzymatische Bildung des Norepinephrins und des Epinephrins wurde als Funktion des pH und der Zusammensetzung der Puffergemische untersucht. Eine maximale enzymatische Aktivität wurde bei einem pH von 5,5 beobachtet. Durch Zusatz von Phosphatpuffer wird die Aktivität gehemmt. Eine maximale Hemmung der enzymatischen Norepinephrinbildung durch DPA und Tryptamin wurde bei einem pH von 5,5 und durch Adrenalon bei einem pH von 6,5 festgestellt. Der Reaktionsmechanismus wurde auf Grund der Ergebnisse erläutert.

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