Summary

A number of basically substituted benzimidazoles show a strong analgesic activity in mice, rats, rabbits, and dogs. Qualitatively they act very similarly to morphine and corresponding synthetic analgetics and the most active of these compounds are quantitatively superior to all analgetics hitherto described.

Kinetics of Penicillinase Activity

Penicillinase, next to catalase, has one of the highest turnover numbers known ($1{\cdot}48 \times 10^5$ M of substrate hydrolysed per min at 30°)[1]. It is of interest then to determine whether MICHAELIS-MENTEN kinetics applies in this instance.

Rate of hydrolysis of benzyl penicillin by penicillinase from *B. subtilis* (commercial preparation; Commonwealth Serum Laboratories, Australia) has been investigated by a slight modification of the method of WISE and TWIGG[2]. Preliminary experiments having shown that the effect of potassium chloride in the range of concentration 0·002–0·08 M on the rate was negligible, a constant ionic strength of 0·02 maintained by potassium chloride was used in the present investigation.

Results indicate that the reaction follows MICHAELIS-MENTEN kinetics[3], at least between concentrations of substrate 20 and 0·2 times the apparent MICHAELIS constant K_m'. This is evident from a plot of

$$\frac{(t+[S])}{V'} \text{ against } \log[S].$$

(Fig. 1; where t is the time in minutes, $[S]$ is the concentration of penicillin remaining, and V' is the apparent maximum [zero order] velocity expressed as mole per min · 10^5, at 25° C, pH 6·58 and ionic strength 0·02.)

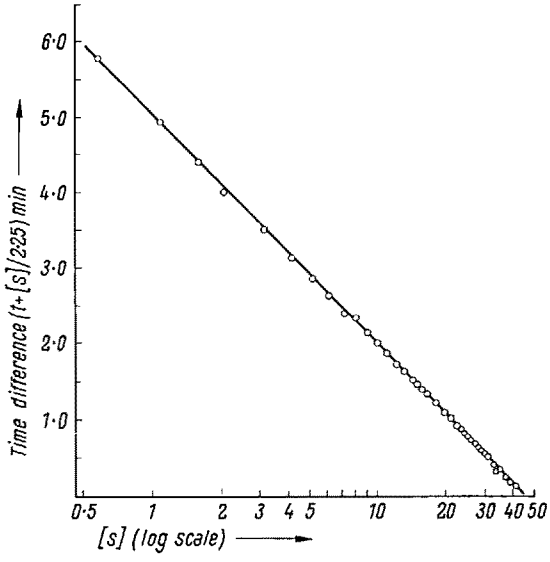

Fig. 1.
Time difference plot (see text for explanation).

Linearity of the time difference

$$(t + [S] \div V' + \text{constant};$$

i.e. actual time, less time calculated for zero order kinetics) with log $[S]$ is in accord with the relationship

$$-V' \cdot t = [S] + K_m' \ln[S] + \text{constant}$$

obtained on integration of the MICHAELIS-MENTEN rate equation[3].

Variation with pH of the parameters V' and K_m', estimated by LINEWEAVER-BURK plots[4] and corrected for the second dissociation constant of penicilloic acid $pK_{A,2}$ 5·35[5], is summarised in Figure 2. These results are

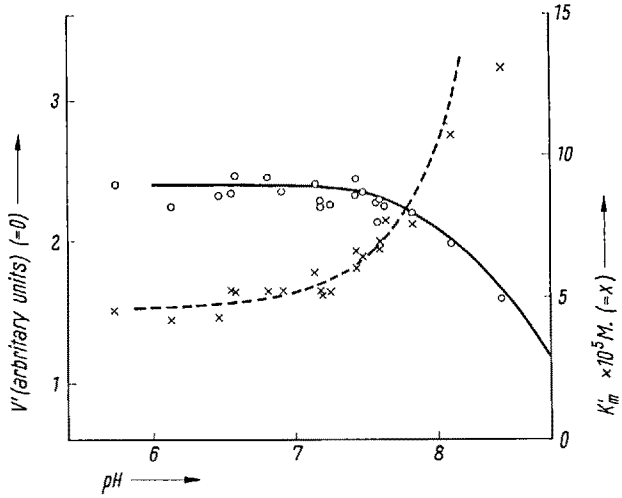

Fig. 2.
Variation of the parameters V' and Km' with pH.

consistent with competitive inhibition of the enzyme by hydroxyl ions involving functional groups of $pK_A \sim 7{\cdot}9$, and with anti-competitive inhibition[6] involving functional groups of $pK_A \sim 8{\cdot}8$. Curves shown in the Figure represent the expected variation of the parameters calculated[7] on this basis.

At pH 7·14 and in the temperature range 22° to 43° C, the parameters V' and K_m' show a variation with temperature in accord with the ARRHENIUS equation[8]:

$$V' = A\, e^{\frac{-5700}{RT}}$$

and

$$K_m' = 1{\cdot}46 \times 10^{-4}\, e^{\frac{-700}{RT}} \quad M$$

where $R = 1{\cdot}99$ cal per degree, and A is a constant. A slight deviation observed at 47° may be due to partial inactivation of the enzyme under these conditions.

Variation of the parameters K_m' and V' were less than the experimental error either for a threefold dilution of the enzyme or for change in concentration of the reaction products in the range 3×10^{-4}–3×10^{-3} M.

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[5] R. MOZINGO and K. FOLKERS, *The Chemistry of Penicillin* (editors H. T. CLARKE, J. R. JOHNSON, and R. ROBINSON, Princeton, University Press, 1949), p. 573.
[6] W.A. RAWLINSON, Rev. pure appl. Chem. *6*, 110 (1956).
[7] W.A. RAWLINSON, Rev. pure appl. Chem. *6*, 118 (1956).
[8] S. ARRHENIUS, Z. physical. Chem. *4*, 226 (1889).

Variation of V' with pH (Fig. 2) is reasonably in accord with the results of POLLOCK *et al.*[9], who found the greatest zero order velocity to occur in the region of pH 6, but differs considerably from that found by earlier workers. It should be noted that failure to take account of singly ionised penicilloic acid (pK_A 5·35[5]) may introduce considerable error at pH values below 7 when an alkalimetric or manometric method is employed. For example, the uncorrected results of the present investigation led to a spurious flat topped maximum for V' between pH values 6·6 and 7·6. This may account in part for some discrepancies in the literature; maxima have been variously reported at pH 7·8[1], and 6·9–7·6[10], for example. In the latter case (manometric method) the flat topped curve described appears little different from the plot of uncorrected V' values obtained in the present investigation.

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Zusammenfassung

Die Kinetik der Penicillinhydrolyse durch Penicillinase aus *B. subtilis* ist mittels einer titrimetrischen Methode verfolgt und in Übereinstimmung mit der Gleichung von MICHAELIS und MENTEN befunden worden. Die pH-Abhängigkeit wird diskutiert.

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[10] R. J. HENRY and R. D. HOUSEWRIGHT, J. biol. Chem. *167*, 559 (1947).

Biochemical Predetermination in *Drosophila*

In their paper chromatographic study of the development of *Drosophila melanogaster*, HADORN and MITCHELL[1] demonstrated that the eggs contained a blue fluorescent substance referred to as Fl 6. The material disappeared at the beginning of larval stages and did not reappear until the end of larval life. In young adults Fl 6 was clearly demonstrated only in females. No other fluorescent materials were found in the *Drosophila* eggs. In *Ephestia kühniella* the eggs have been shown to contain several fluorescent compounds[2]. DANNEEL and ZIMMERMANN found that the Fl 6 of HADORN and MITCHELL is kynurenine[3]. As would be expected, kynurenine was not found in *vermilion* (*v*) flies, since this mutant is unable to carry out the synthesis of kynurenine from tryptophane[4].

The classical example of predetermination in *Ephestia* is believed to be due to the deposition of kynurenine or is some related compound in the eggs of kynurenine-containing females[5]. Thus the offspring of these females can form the ommochrome pigments derived from kynurenine regardless of their genotypically induced inability to synthesize kynurenine. Maternal inheritance has also been demonstrated with regard to the egg pigments of the silkworm, *Bombyx mori*[6]. By means of paper chromatography and fluorescence measurements, kynurenine metabolism can readily be studied quantitatively despite the absence of visible pigments in *Drosophila* eggs. Therefore it appeared to be of some interest to investigate possible maternal effects in *Drosophila melanogaster*.

Kynurenine in 200 eggs of various matings. The amount of kynurenine is expressed in terms of arbitrary units of fluorescence.

Mating	*n*	Fluorescence
$+ + \times +$	6	$120 \pm 3{,}0$
$+ v \times v$	6	$110 \pm 5{,}0$
$v v \times +$	6	$3{,}3 \pm 1{,}1$
$v v \times v$	6	$1{,}5 \pm 1{,}2$

Eggs from a vermilion strain clearly lacked kynurenine even when 400 eggs were used for a single chromatogram. With wild type flies of the strain Sevelen, a spot corresponding in Rf and fluorescence with kynurenine could readily be demonstrated with 50 eggs. The eggs were collected, from standard food containing yeast, with a needle and squashed with a glass rod directly on the paper for chromatography. No special steps were taken to free the eggs from the yeast with which they were contaminated since chromatography of yeast alone showed no fluorescent spots which would interfere with measurements of kynurenine. Ascending chromatograms were prepared using propanol–1% aqueous ammonia (2:1) as the developing solvent. In experiments involving fluorescent measurements, 200 eggs 1 ± 1 h old were used. After the solvent front had moved ca. 22 cm the chromatograms were removed and dried at room temperature. Chromatograms were examined for fluorescence with a long wave length UV lamp. Measurements were made directly on the paper using the method of HADORN and KÜHN[7]. Eggs produced by the matings $+v \times v$ and $vv \times +$ were compared with each other and with homozygous + and *v* eggs. Both the above-mentioned matings are expected to produce 50% + containing eggs although obviously different with regard to sex and heterozygosity.

The results of the fluorescence measurements are shown in the Table. The data indicate that the kynurenine content of the eggs is determined by the genotype of the mother. No kynurenine could be detected in the eggs of a *v* female crossed with a + male, regardless of the fact that all female eggs were now heterozygous for +. This was also found to be true within the limits of the technique with 200 eggs from the same cross 7 ± 1 h old. The kynurenine which is present in 1 h eggs from + mothers gradually disappears and by 17 h can barely be detected on the chromatograms. The values for the crosses $vv \times +$ and $vv \times v$ in the Table are probably due to background fluorescence and do not demonstrate the presence of any kynurenine.

These investigations seem to indicate that kynurenine accumulates in the ovaries of *Drosophila* and is deposited in the eggs. Chromatograms of the ovaries from mature females reveal large amounts of this substance. It is not

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