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Kinetic Studies of the Metabolism of Foreign Organic Compounds

5. A MATHEMATICAL MODEL EXPRESSING THE METABOLIC FATE OF PHENOLS, BENZOIC ACIDS AND THEIR PRECURSORS

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Previous papers from this laboratory have recorded the results of studies of the kinetics of the reactions which benzoic acid, phenols and compounds which give rise to them, undergo in the rabbit (Bray, Thorpe & White, 1951; Bray, Humphris, Thorpe, White & Wood, 1952*a, b, c*). These results have provided a basis for the construction of a mathematical model expressing the quantitative fate of such compounds. This model is in accord with experimental results obtained previously both in this and other laboratories. Experiments with aniline, acetanilide, *o*- and *p*-aminophenols, *o*- and *p*-hydroxyacetanilides and *o*-, *m*- and *p*-hydroxybenzaldehydes have been carried out as further tests of the validity of the model.

THEORETICAL

In this section mathematical expressions are derived representing the fate of compounds such as phenols, benzoic acids and their precursors undergoing metabolic reactions according to a defined pattern of kinetics.

In the previous paper (Bray *et al.* 1952*c*) it was shown that for small doses of a phenol the rate of conjugation with sulphate is proportional to the body level of the phenol, while for large doses the rate is constant and equal to the maximum rate of supply of sulphate ion. In the present paper it will be assumed that the transition from first-order to zero-order kinetics is abrupt. Thus, with increasing body level of the phenol, the rate of conjugation with sulphate increases in proportion until the maximum rate at which sulphate can be supplied is reached.

The magnitude of this critical body level will be fixed by the values of the first- and zero-order velocity constants for conjugation. Although such transitions are not encountered in purely chemical systems, our experimental results suggest that they may occur in the rabbit. The mechanism seems to be related to the tendency to maintain a certain blood level of sulphate ion, essentially by means of the kidney. Similar kinetic considerations appear to apply also to glycine conjugation.

The basic mathematical treatment of such a system has already been presented for benzoic acid (Bray *et al.* 1951). In this the critical body level of benzoic acid was equated to zero since the very high value of the first-order velocity constant for glycine conjugation rendered the critical value negligibly small. This treatment is now extended to represent the metabolism of compounds for which this approximation cannot be made. The substance formed, actually or theoretically from the precursor, whether it be a phenol, a benzoic acid or some other compound, is described as the intermediate.

Symbols

A = dose level of precursor; A_{crit} = critical dose level of precursor; B = dose level of intermediate; B_{crit} = critical dose level of intermediate; b = amount of intermediate, free and conjugated, at time t ; g = amount of glucuronide at time t ; s = amount of second conjugate at time t (e.g. an ethereal sulphate for phenols, a hippuric acid for benzoic acids); $m = g + s$; T = time at which critical body level of free intermediate is reached; k_1 = first-order velocity constant for conversion of precursor

to intermediate; k_g = first-order velocity constant for glucuronide conjugation; k_s = first-order velocity constant for second conjugation; $k_m = k_g + k_s$; v_s = zero-order velocity constant for second conjugation; G = total amount of glucuronide formed; S = total amount of sulphate formed.

Most of these symbols were used in the study of the kinetics of the conjugation of benzoic acid (Bray *et al.* 1951). The more general symbol v_s now denotes zero-order velocity constant for the second conjugation in preference to the k_h formerly used in reference to hippuric acid formation. Equations 3, 7 and 9 from that paper (p. 95) are quoted using the revised symbols:

$$b = B + A(1 - e^{-k_b t}), \quad (1)$$

$$m = \left(A + B + \frac{v_s}{k_g} \right) (1 - e^{-k_g t}) + \frac{k_g A}{k_g - k_b} (e^{-k_g t} - e^{-k_b t}), \quad (2)$$

$$g_T = A(1 - e^{-k_b T}) + B - v_s T. \quad (3)$$

These equations will now be generalized. When the dose level of the precursor administered is less than a certain value ($A_{\text{crit.}}$) the body level of the free intermediate compound will be less than $(b - m)_{\text{crit.}}$ and the rate of production of the two conjugates will be in the ratio of the respective first-order velocity constants for the conjugation processes. The amount of glucuronide produced will be

$$g = k_g m / k_m,$$

$$\text{and when } t = \infty \quad G = k_g A / k_m,$$

providing these are the only reactions taking place. At the critical body level of the unconjugated intermediate, i.e. at time T , the rate of formation of the second conjugate is given by the equation

$$ds/dt = v_s = k_s(b - m)_{\text{crit.}} = k_s(b_T - m_T),$$

since the body level of unconjugated intermediate at any time is given by the expression $(b - m)$.

Now the general expression for the total amount of glucuronide formed may be derived using the condition that at time T

$$b_T - m_T = v_s / k_s.$$

The glucuronide formed between $t = 0$ and $t = T$ is given by

$$[A(1 - e^{-k_b T}) + B - v_s T] - \frac{v_s}{k_s}$$

and that formed after $t = T$ is given by

$$\frac{k_g}{k_m} \left(A e^{-k_b T} + \frac{v_s}{k_s} \right).$$

Thus the total glucuronide formed is given by

$$G = [A(1 - e^{-k_b T}) + B - v_s T] - \frac{v_s}{k_s} + \frac{k_g}{k_m} \left(A e^{-k_b T} + \frac{v_s}{k_s} \right)$$

which, on rearranging and substituting, gives

$$G = A + B - v_s T - \frac{k_s}{k_m} \left(A e^{-k_b T} + \frac{v_s}{k_s} \right), \quad (4)$$

and the total second conjugate formed is given by

$$S = v_s T + \frac{k_s}{k_m} \left(A e^{-k_b T} + \frac{v_s}{k_s} \right). \quad (5)$$

Equation 2 can be extended to represent the metabolism of compounds which are excreted to some extent unconjugated, or which contain substituent groups which undergo modification, if these secondary processes follow first-order reaction kinetics. Where the precursor is excreted unchanged or contains a substituent group which is modified or undergoes any reaction other than the primary reactions considered here, k_b and k_g must be replaced by other first-order velocity constants which comprise the first-order velocity constants of all the individual reactions.

It can be calculated that the critical dose level of a precursor is given by

$$A_{\text{crit.}} = \frac{v_s}{k_s} \left[\frac{k_m}{k_b} \right] \left(\frac{k_m}{k_m - k_b} \right) \quad (6)$$

and of its intermediate by

$$B_{\text{crit.}} = v_s / k_s, \quad (7)$$

assuming instantaneous absorption in both cases.

When equation 2 is simplified to represent the metabolic fate only of an administered precursor, it is not completely accurate, since it is assumed that, for dose levels above $A_{\text{crit.}}$, the body level of the intermediate rises immediately above the critical body level, whereas in fact this increase from zero body level is not instantaneous. While it would be possible to derive the appropriate, more complicated, mathematical expression, in practice this is not necessary, since, for the compounds considered, the increase occupies only a relatively short time and the correction is in any case smaller than that which must be applied to allow for a finite absorption time. If absorption occupies a finite time, the maximum body level of the intermediate which can be attained will be lower, so that the critical dose levels will be somewhat higher than those defined above, and, in particular, the critical dose level, $B_{\text{crit.}}$, of the intermediate will no longer be identical with the critical body level, $(b - m)_{\text{crit.}}$. The correction is, however, relatively small and, for simplicity, the above equations will be applied throughout this paper.

EXPERIMENTAL

The experiments were of the type described previously (Bray *et al.* 1951). The compound under investigation was administered to a fasting rabbit and the urine samples passed during the period of elimination of the dose collected and analysed individually by the appropriate methods previously described (Bray *et al.* 1952a). The compounds studied were aniline, acetanilide, *o*- and *p*-aminophenols and *o*- and *p*-hydroxyacetanilides. Results from these experiments are included in Table 1. Similar experiments

Table 1. *The percentage of the dose of various phenols and phenol precursors excreted as glucuronide and ethereal sulphate by the rabbit (unless stated otherwise)*

Compound	Dose level (g./kg.)	% excreted as		G/S	Remarks
		Glucuronide G	Ethereal sulphate S		
Group 1					
Benzene	0.5 ⁽¹⁾	11	10	1.1	39% excreted unchanged in expired air ⁽²⁾
Phenol	0.25 ⁽¹⁾	70	19	3.7	$k_g = 0.56 \text{ hr.}^{-1}$
	0.20 ⁽³⁾	69	15	4.6	
Catechol	0.1 ⁽⁴⁾	70	18	3.9	—
	0.2 ⁽³⁾	76	18	4.2	—
Quinol	0.15 ⁽⁴⁾	43	30	1.4	—
	0.2 ⁽³⁾	78	18	4.3	Administered to fasting animal
Chlorobenzene	0.15 ⁽⁵⁾	25	27	0.9	—
<i>o</i> -Chlorophenol	0.17 ⁽⁵⁾	82	19	4.4	—
<i>p</i> -Chlorophenol	0.17 ⁽⁵⁾	88	13	6.9	—
	0.23 ⁽³⁾	81	11	7.4	—
4-Chlorocatechol	0.15 ⁽⁶⁾	87	15	5.7	—
Anisole	0.3 ⁽³⁾	48	29	1.7	$k_b = 0.11 \text{ hr.}^{-1}$
<i>p</i> -Methoxyphenol	0.38 ⁽³⁾	69	13	5.3	$k_g = 0.54 \text{ hr.}^{-1}$
Aniline	0.07 ⁽⁷⁾	0	100	0	Rats
	0.2 ⁽⁸⁾	70	28	2.5	—
	0.2 ⁽⁹⁾	59	20	2.8	—
	0.2 ⁽⁹⁾	68	36	1.9	Sulphite given with dose. $k_b = 0.27 \text{ hr.}^{-1}$
<i>o</i> -Aminophenol	0.21 ⁽⁸⁾	42	25	1.7	—
	0.25 ⁽⁹⁾	60	22	2.7	—
<i>p</i> -Aminophenol	0.9 ⁽⁷⁾	100	0	∞	Rats
	0.25 ⁽⁸⁾	43	18	2.5	—
	0.25 ⁽⁹⁾	55	18	5.5	Administered to fasting animal
Group 2					
Phenylurea	0.2 ⁽⁹⁾	39	25	1.6	—
<i>N</i> -(<i>p</i> -Hydroxyphenyl)-urea	0.2 ⁽⁹⁾	34	25	1.4	$k_g = 0.11 \text{ hr.}^{-1}$
Benzoxazolone	0.2 ⁽⁹⁾	53	26	1.7	$k_b = 0.29 \text{ hr.}^{-1}$
Hydroxybenzoxazolone	0.2 ⁽⁹⁾	43	28	1.9	$k_g = 0.18 \text{ hr.}^{-1}$
Acetanilide	0.25 ⁽⁸⁾	70	12	5.8	—
	0.25 ⁽⁹⁾	58	10	5.8	$k_b = 0.43 \text{ hr.}^{-1}$
	0.25 ⁽⁹⁾	80	29	2.7	Sulphite given with dose
<i>o</i> -Hydroxyacetanilide	0.28 ⁽⁸⁾	39	20	2.0	—
	0.25 ⁽⁹⁾	50	22	2.3	—
<i>p</i> -Hydroxyacetanilide	0.28 ⁽⁸⁾	63	10	6.3	—
	0.25 ⁽⁹⁾	81	6	13.5	Administered to fasting animal
	0.25 ⁽⁹⁾	77	6	12.8	$k_g = 0.50 \text{ hr.}^{-1}$

(1) Porteous & Williams, 1949.

(2) Parke & Williams, 1950.

(3) Bray *et al.* 1952*a, b, c.*

(4) Garton & Williams, 1949.

(5) Spencer & Williams, 1950.

(6) Dodgson & Williams, 1949.

(7) Elson *et al.* 1946.

(8) Smith & Williams, 1949.

(9) This paper.

p-Hydroxybenzenesulphonamide has been omitted from group 2 since its theoretical precursor, benzenesulphonamide, is hydroxylated to such a small extent in the rabbit that values for glucuronide and ethereal sulphate would be unreliable.

Table 2. *Excretion products of o-, m- and p-hydroxybenzaldehydes in the rabbit*

(Dose level 0.4 g./kg. Ranges given in parentheses and number of experiments as superior figures.)

	% of dose excreted as			
	Ether-soluble acid	Ester glucuronide	Ether glucuronide	Ethereal sulphate
<i>o</i> -Hydroxybenzaldehyde	75 (64-81) ³	18 (9-29) ⁶	9 (0-19) ⁶	3 (2-4) ³
<i>m</i> -Hydroxybenzaldehyde	75 (66-90) ⁶	9 (8-9) ⁶	9 (2-13) ⁶	7 (6-9) ³
<i>p</i> -Hydroxybenzaldehyde	67 (51-86) ³	4 (0-6) ⁸	16 (10-21) ⁸	9 (9-10) ³

were carried out on human subjects using benzoic acid. Some further experiments with 24 hr. samples of urine were also carried out with *o*-, *m*- and *p*-hydroxybenzaldehydes, using the analytical methods described by Bray, Ryman & Thorpe (1947). The results obtained are given in Table 2.

DISCUSSION

The mathematical model gives quantitative values for the proportions of the dose excreted as the appropriate metabolites in terms of reaction velocity constants and the dose level. The model reveals certain characteristic features of the metabolic system which may be compared with experimental data. For much of the data in the literature comparison must be qualitative, but, where velocity constants have been evaluated by investigations of the kinetics, comparisons can be made quantitative. In the following sections experimental data from kinetic studies reported in this and previous papers and from 24 hr. investigations in this and other laboratories are considered in relation to the mathematical model.

Variation in dose level

When an administered compound is excreted only as two conjugates, the proportions of the dose appearing in the two forms are complementary.

Table 3. Variation in ethereal sulphate excretion by rabbits after administration of phenol and *p*-hydroxybenzenesulphonamide at different dose levels

Compound	Dose level (mg./kg.)	Percentage of dose excreted as ethereal sulphate	
		Found*	Calculated†
Phenol	25	41	37
	50	37	28
	100	18	20
	200	18	13
	500	19	11
<i>p</i> -Hydroxybenzene-sulphonamide	100	53	56
	250	44	43
	500	33	33
	750	27	23

* Values for phenol given by Williams (1938) and for *p*-hydroxybenzenesulphonamide by Sammons *et al.* (1941).

† Uncorrected for absorption time.

Equations 4 and 5 show that these proportions are a function of the dose level (*A* and/or *B*) for dose levels above the critical values $A_{crit.}$ and $B_{crit.}$ (equations 6 and 7). The proportion excreted as that conjugate formed only by a first-order reaction (glucuronide) increases with increasing dose level, while that formed by a zero-order reaction (ethereal sulphate, hippuric acid) decreases. It is most convenient to consider the conjugate excreted in

smaller amount, since this shows a greater relative variation.

We have already shown that the proportion of a dose of benzoic acid excreted as benzoylglucuronide is greater for higher dose levels (Bray *et al.* 1951). Variation in the extent of ethereal sulphate formation at different dose levels was recorded for phenol by Williams (1938) and for *p*-hydroxybenzenesulphonamide by Sammons, Shelswell & Williams (1941). These data are compared in Table 3 with the values calculated by means of equation 5, using values of constants (v_s , k_g , k_s) derived experimentally for the rabbit in previous kinetic studies (Bray *et al.* 1952*b*, *c*), i.e. $v_s = 4.5$ mg. $SO_3/kg./hr.$; for phenol $k_g = 0.56$ hr. $^{-1}$, $k_s = 0.35$ hr. $^{-1}$; for *p*-hydroxybenzenesulphonamide $k_g = 0.16$ hr. $^{-1}$, $k_s = 2.1$ hr. $^{-1}$. It is clear that the observed effects of varying the dose levels are close to those calculated from equation 5.

Effect of administration as precursor

An intermediate, e.g. a phenol or a benzoic acid, gives rise to excretion products which are the same whether it is administered as such or formed *in vivo* from a precursor, but the proportions of the metabolites usually differ considerably for equimolecular doses. For dose levels below the critical ones the proportions of the intermediate excreted as different conjugates do not vary. The critical dose for the intermediate ($B_{crit.}$) is defined in equation 7, and for the precursor ($A_{crit.}$) in equation 6. For dose levels above $A_{crit.}$ and $B_{crit.}$ the considerations discussed in the previous section apply. It so happens that for most precursors and their intermediates, the quantitative metabolic fate of which has been determined, the order of dose level low enough to be without obvious toxic effect and yet high enough to permit accurate estimation of excretion products greatly exceeds $B_{crit.}$ and is comparable with $A_{crit.}$. When this condition holds, the ratio of the amount of glucuronide to the amount of the other conjugate will be smaller if the precursor is administered than if the intermediate is administered.

Although we have not yet investigated the kinetics of the metabolic reactions of substituted benzoic acids and their precursors in detail, there are indications from other studies that the same mathematical model is applicable to certain of these compounds. Administration of the chloro- and fluoro-benzoic acids (Bray, Clowes, Thorpe, White & Wood, 1952) gives rise to the excretion of a considerable proportion of the dose as unconjugated acid, but it was shown that the ratio of the proportion of the dose excreted as the glycine conjugate to that excreted as glucuronide was greater after administration of the amide than after the acid. That the ratio is greater for amide than for acid is a characteristic of the model, whether the rate of excretion of

unconjugated acid increases or decreases with decreasing body level, while the relation between the percentages of a metabolite excreted is only characteristic if the excretion of unconjugated acid follows first-order kinetics. We have not investigated in any detail the relation between rate of excretion of an unconjugated compound and its body level, but have noted that the rate usually decreases with decreasing body level, and may, therefore, be considered to follow, approximately, first-order reaction kinetics. When little excretion of the unconjugated intermediate occurs, variations in the proportion of the dose excreted as the minor conjugate forms the most sensitive index for comparison. This is seen in the results of a study of *m*- and *p*-methyl-substituted toluenes, benzamides and benzoic acids (Bray, Humphris & Thorpe, 1949; Bray, Thorpe & Wood, 1949). The percentage of doses of these compounds excreted as *m*- and *p*-toluyglucuronides were, for the *meta* isomers, 2, 9 and 26 respectively, and, for the *para* isomers, 1, 4 and 14. These figures are compatible with equation 4 and show that the methyl group is converted more slowly than the carbamyl group, as was found for toluene and benzamide (Bray *et al.* 1951).

Data from the literature relating to the excretion of ethereal sulphate and glucuronide after administration of various phenols and phenol precursors are summarized in Table 1, in which the compounds have been placed in two groups on the same basis as previously (Bray *et al.* 1952*b, c*), i.e. compounds in group 1 have small substituent radicals.

It can be seen that for compounds of group 1 (benzene, chloro-, amino- and methoxy-benzenes and their metabolic hydroxylation products) the value of the ratio G/S (the ratio of the percentages of the dose excreted as glucuronide to that excreted as sulphate) for the precursors is smaller than for the phenols. Apparent exceptions are the findings of Garton & Williams (1949) for quinol (a hydroxylation product of benzene) and of Smith & Williams (1949) for *p*-aminophenol (a hydroxylation product of aniline). Their values for G/S are of the same order as those for benzene and aniline, respectively, whereas we found that these phenols, like the other phenols of group 1, gave values for G/S of approximately 5. As will be seen later (Table 4), this difference cannot be attributed to the slightly different dose levels used, but may be due in part to the fact that the animals used in our experiments were fasting. The results of Garton & Williams might be expected if their rabbits ingested their daily food ration at a time close to that of dosage. The effect of diet and time of feeding is discussed in a later section.

Those phenols of group 1 which have been investigated in detail have values for k_e and k_g which are close to those of phenol itself (Bray *et al.*

1952*b, c*). The values of k_e for the precursors in group 1 are low. That for anisole is 0.11 hr.⁻¹; benzene (Porteous & Williams, 1949) and chlorobenzene (Spencer & Williams, 1950) are excreted at least as slowly as anisole. For compounds of group 1 the values of G/S for phenols and precursors at the dose levels employed must be widely different, and small variations in the dose levels will not obscure this difference. This can be seen in Table 4 which shows the values of G/S calculated using equations 4 and 5 at different dose levels of anisole and *p*-methoxyphenol, compounds for which the values of the appropriate constants are known.

Table 4. Calculated values for G/S after administration of anisole and *p*-methoxyphenol to rabbits at varying dose levels

Dose level (g./kg.)	Anisole	<i>p</i> -Methoxyphenol
0.1	1.0	3.5
0.2	1.2	5.2
0.3	1.7	7.1
0.4	2.2	8.5
0.5	2.7	10.0

The values for aniline and *p*-aminophenol in the rat given by Elson, Goulden & Warren (1946) require comment. These authors stated that 'aniline is excreted exclusively as ethereal sulphate, whereas the equivalent dose of *p*-aminophenol is excreted entirely as glucuronate'. In view of this they suggested that ethereal sulphate could be formed in the rat 'directly in the aromatic nucleus without preliminary formation of free phenol compounds'. It seems unlikely that there is a qualitative difference between the metabolism of phenols in the rabbit and rat, although quantitative differences might be expected. We have found, for example, that in the rabbit the value of G/S for a small dose of *p*-hydroxybenzenesulphonamide (0.2 g./kg.) is 0.11, while for a large dose (2 g./kg.) it is 7.9. When repeated very small doses (0.018 g./kg./hr.) are administered together with sodium sulphite, a method of dosage approximately corresponding to the slow formation of the phenol from a precursor *in vivo*, the amount of glucuronide is not detectable. In the rabbit the value of k_e for *p*-hydroxybenzenesulphonamide is considerably greater than that of k_g . It is reasonable to suggest that the results of Elson *et al.* are of this type and that they failed to detect the minor metabolites due to limitations of their experimental methods. If this explanation of their results is correct their inference of direct sulphation of aniline is no longer valid.

For the phenols included in group 2 of Table 1, k_g is small and the value of G/S increases only slightly as the dose level increases, so that there is little difference between the values of the ratio for phenol

and precursor. For instance, the calculated values of G/S for phenylurea and *p*-hydroxyphenylurea at the dose levels cited are 1.3 and 1.6 respectively. The experimental results are therefore as would be expected.

Acetanilide appears to differ from all the precursors considered so far, in that the value of G/S is unusually high for the dose level cited. This is not because the value of k_e for *p*-hydroxyacetanilide is abnormally low, since the simultaneous administration of a sulphate precursor with acetanilide reduces the value of G/S to a more usual value (2.7), while the value of k_e (0.5 hr.^{-1}) is of the same order as for

methoxyphenol assuming instantaneous absorption. The values of constants substituted in equations 1 and 2 for this calculation are as follows:

$$v_s = 4.5 \text{ mg. SO}_3/\text{kg./hr.}; \quad k_b = 0.11 \text{ hr.}^{-1}; \\ k_e = 0.54 \text{ hr.}^{-1}; \quad k_s = 0.54 \text{ hr.}^{-1}.$$

Thus the magnitude of the dose of an intermediate having the same metabolic fate as a given dose of its precursor (in the fasting animal) is not the equimolecular dose. For instance, the dose of *p*-methoxyphenol having the same metabolic fate as 1.15 g. of anisole is not 1.0 g. but 0.17 g. of *p*-methoxyphenol.

Species differences

Quantitative differences may occur in the metabolic fate of a given compound in different species, even when the metabolites are identical. The velocity constants found in these kinetic studies must comprise both fundamental chemical constants and parameters characteristic of the organism. While the chemical constants of the enzyme system might be assumed to be identical in all species, the parameters relating to factors approximately constant within a species (e.g. amount or concentration of enzyme) may differ. Thus a reaction may follow similar kinetics in different species, the quantitative differences being due to variations in the values of velocity constants (e.g. the metabolism of aniline and *p*-aminophenol in rabbit and rat, p. 427). In a previous paper (Bray *et al.* 1951) the different proportions of glucuronide excreted by the rabbit, dog, pig and man in response to a dose of benzoic acid were related to the rates of availability of glycine (i.e. the zero-order velocity constant for the formation of hippuric acid) in the species. In these calculations it was assumed that the first-order velocity constant for glucuronide formation (k_e) was the same for all four species, and it was shown that the variation in glycine availability was sufficient to account for species difference together with the dose-level effect, since benzoic acid was given to man at a much lower dose level than to the other species. Approximate values for k_e have now been calculated for the dog and the pig using data of Quick (1931) and Csonka (1924). These are 0.09 and 0.06 hr.^{-1} respectively. The value for the rabbit is 0.08 hr.^{-1} (Bray *et al.* 1951). Experiments on two human subjects gave 0.03 hr.^{-1} , a value which differs appreciably from that for the rabbit. Substitution of this value in equation 3 gives a value of 4 for the percentage of a dose of benzoic acid excreted as glucuronide (dose level 0.043 g./kg.). This compares more favourably with the value of 5, found experimentally, than with the value of 9% previously calculated (Bray *et al.* 1951, Table 2).

Fig. 1. Calculated body level of *p*-methoxyphenol resulting from the administration of anisole (0.3 g./kg.) and of *p*-methoxyphenol (0.34 g./kg.) to the rabbit, assuming instantaneous absorption. A-A, anisole; B-B, *p*-methoxyphenol.

the phenols of group 1. The high value of G/S is due to the high value of k_e (0.43 hr.^{-1}). This has the effect of reducing the critical dose level so that $A_{crit.}$ is much lower than the dose level used. Since k_e is high, the value of G/S for both acetanilide and *p*-hydroxyacetanilide will increase greatly with increasing dose level, and the actual value of G/S for both compounds will be relatively high. Large variations in the higher values of G/S can occur through small variations in the value of v_s , so that different large values of the ratio for a compound may be expected. The data for acetanilide and *p*-hydroxyacetanilide thus conform to the model.

From the results quoted in this and the preceding paper (Bray *et al.* 1952c) it is clear that the administration of a precursor is equivalent to the continuous administration of its hydroxylation product at a low dose level. This is illustrated in Fig. 1 which shows the calculated variations in the body level of *p*-methoxyphenol resulting from the administration of 0.3 g./kg. anisole and of 0.34 g./kg. *p*-

Effect of substituents

If a substituent is introduced into the benzene nucleus of an aromatic compound, the metabolic fate of the substituted compound may differ from that of the parent compound since (1) the velocity constants of metabolic reactions involving groups in the parent compound may be different in the substituted compound; (2) the substituent group may itself be modified; (3) the rate of excretion (usually by the kidney) may be different for the two compounds. The relative contributions of these three factors cannot be assessed when the only data available are those derived from a study of 24 hr. urine specimens (i.e. the percentages of the dose excreted as different metabolites). The theoretical value of such results is limited unless they can be interpreted with the help of kinetic data. The greater experimental difficulties in the kinetic method are outweighed by the greater scope in interpretation of the results obtained. As an example some early observations on ethereal sulphate formation carried out in this laboratory (Williams, 1938) may be given a different interpretation. Williams studied the effect of various substituents upon the extent of the sulphate conjugation of phenol in the rabbit. With phenol itself 20% of the dose was conjugated as sulphate, but with eleven *para*-substituted phenols, values ranging from 7 to 33% of the dose were obtained. (Most of these values have been confirmed subsequently by other workers in this laboratory.) The deviations from 20%, the value for unsubstituted phenol, were attributed by Williams to the influence of the substituent group upon sulphate conjugation. For phenols substituted with inert groups in the *para* position, we have shown that sulphate conjugation normally takes place at a uniform rate, and that the proportion of a dose of a phenol excreted as ethereal sulphate depends primarily upon the value of k_e for dose levels of the order of 0.25 g./kg. (Bray *et al.* 1952*b*). There is no reason to suppose that this applies only to inert substituents since *p*-hydroxybenzamide, the carbamyl group of which is hydrolysed to some extent, behaves similarly. For phenols with substituent groups which are extensively modified in the body the extent of sulphate conjugation will depend not only on the k_e for the administered phenol (and its phenolic metabolites), but also on the rates of reactions by which the substituent groups are modified. Some of the compounds showing the greatest departure in the extent of sulphate conjugation from that of phenol itself may now be considered. Only 7% of a dose of *p*-hydroxybenzoic acid forms ethereal sulphate. This is probably due to the fact that the acid itself is readily excreted by the kidney rather than to influence of the carboxyl group upon sulphate conjugation. The substitution

of benzoic acid with chloro and fluoro radicals reduces the total conjugation (Bray *et al.* 1952) probably for the same reason. Williams found that only 12% of a dose of *p*-hydroxybenzaldehyde formed ethereal sulphate. This need not be attributed to the direct influence of the aldehyde group upon the sulphate conjugation process, since *p*-hydroxybenzaldehyde, which would be expected to behave like phenol itself, is largely metabolized to *p*-hydroxybenzoic acid (Table 2). Of the phenols examined by Williams, *p*-hydroxybenzamide formed the greatest proportion of ethereal sulphate (33% of the dose). Such a high percentage is a necessary consequence of the low value for k_e (0.23 hr.⁻¹, Bray *et al.* 1952*b*). The value for ethereal sulphate formed from this compound calculated from equation 5 is 30%. These examples show that the variations in sulphate conjugation may be due to the influence of the substituent group on glucuronide synthesis and not on ethereal sulphate synthesis, to metabolic modification of the substituent group or to the readiness of excretion of the substituted phenol. Correct interpretations of metabolic processes are unlikely to be made from data from 24 hr. studies alone.

Effect of diet

When a reaction follows zero-order kinetics and its rate is determined by the availability of the conjugating moiety, then ingestion of a compound giving rise to the latter will increase the rate of the reaction. It is immaterial whether the administered compound is a simple chemical compound or a constituent of the normal diet. Thus the extent of the conjugation in question, and, therefore, also of the complementary conjugations, will depend upon the nature of the diet. Griffith & Lewis (1923) found that the administration of glycine increased the rate of hippuric acid formation in the rabbit. Our own experiments (Bray *et al.* 1951) confirmed this and showed also that the extent of glucuronide conjugation was decreased. Other workers (e.g. Csonka, 1924) have examined the effect of administering various proteins with benzoic acid and found that their efficacy in increasing hippuric acid excretion could be correlated with their glycine content. Since the first-order velocity constant for glycine conjugation appears to be high, this conjugation process may in practice be considered always to follow zero-order kinetics with respect to benzoic acid, and the zero-order velocity constant (v_s) may be equated to the rate of supply of glycine. The metabolic fate of benzoic acid or of its precursors found in different investigations will not necessarily be the same, since the availability of glycine will depend on the type of food given and on the relative times of administration of the dose and ingestion of the normal diet. It should be noted that the time at

which food is supplied is not necessarily related to the time at which it is ingested since anorexia is often one of the first symptoms of toxicity.

The availability of sulphate will also influence the ethereal sulphate conjugation of phenols. If certain sulphur compounds are administered sulphate conjugation can be made to follow first-order reaction kinetics, but the rate of production of sulphate from foodstuffs is so small (see Table 1, Bray *et al.* 1952c) that, except at very low dose levels of a phenol, the reaction follows zero-order kinetics, the zero-order velocity constant (v_0) varying with the nature of the food and the time of its ingestion relative to that of the dose.

SUMMARY

1. A mathematical model is derived for metabolic systems in which a precursor is converted into an

intermediate by a first-order reaction, the intermediate then being conjugated by two processes, one of which is first order, and the other first order below a certain body level and zero order above that level.

2. This model is shown to apply to the metabolic fate of certain benzoic acids and phenols and certain of their precursors.

3. The effects of variation in dose level, nuclear substitution of aromatic compounds, and the composition of the diet are discussed.

4. Various data obtained from 24 hr. studies are discussed in terms of the mathematical model.

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Liver Alcohol Dehydrogenase and Ester Formation

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As a result of his observation of clinical cases of methanol poisoning, Røe (1943) formed the opinion that the toxic effects of methanol in man were minimized by the simultaneous ingestion of ethanol. He suggested that ethanol might act as an antidote by virtue of 'the capacity of ethyl alcohol to displace methyl alcohol from the inner surfaces of cells'. The characteristic toxic effects of methanol have, however, generally been attributed to the action of its oxidation products rather than to methanol itself, and it seemed possible that the

beneficial effect of ethanol might be the result of competitive inhibition of an early enzymic stage in the metabolism of methanol. Experiments carried out on human subjects have shown clearly that the metabolism of methanol can be strongly impeded at its earliest stage by the administration of ethanol, and *in vitro* tests have provided evidence of competitive inhibition by ethanol of the oxidation of methanol to formaldehyde by liver alcohol dehydrogenase (Zatman, 1946; Leaf & Zatman, 1952). The *in vitro* tests referred to were carried out with horse-