completely converted oxaloacetic acid into pyruvic acid. In contrast, extraction of the plants with 2N-NaOH immediately inactivated the decarboxylase and stabilized the oxo acid levels, so that oxaloacetic acid could be reliably estimated. Although our plant extraction procedure involved slightly acid pH values this could not explain the apparent absence of oxaloacetic acid, since extractions using 2N-NaOH also failed to reveal the acid.

SUMMARY

1. The oxo acids present in groundnut plants include α -oxoglutaric, pyruvic, glyoxylic and γ -methylene- α -oxoglutaric acids, together with other unidentified oxo acids. All the acids were characterized chromatographically as their 2:4-dinitrophenylhydrazine derivatives, and by catalytic hydrogenation of the latter to the corresponding amino acids. Oxaloacetic acid has not been detected.

2. The amounts of these acids present in leaves, cotyledons and roots were determined at various stages of seedling growth. Concentrations of individual oxo acids were normally in the range of

0–40 μ g./g. fresh weight of plant tissue, but α -oxoglutaric acid concentration showed a marked increase during the process of leaf ageing.

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Kinetic Studies of the Metabolism of Foreign Organic Compounds

7. TOLUIC ACIDS AND N-TOLYLUREAS*

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Previous papers in this series (Bray, Humphris, Thorpe, White & Wood, 1952*b, c, d*; 1955; Bray, Thorpe & White, 1952*e*) have dealt mainly with the kinetics of reactions which *para*-substituted aromatic compounds undergo in the rabbit. It is known that in the dog and in the rabbit the conjugation reactions of *ortho*-, *meta*- and *para*-isomers are often qualitatively similar (e.g. see Quick, 1932*a, b*; Thorpe, 1950), although quantitatively there are gradations in the percentage of a dose conjugated corresponding to the position of the substituent. For example, the toluic acids are excreted by the rabbit mainly as conjugates of glucuronic acid and glycine; the amount of the former increases progressively from the *para*- to the *ortho*-isomer and that of the latter from *ortho* to *para* (Bray, Thorpe & Wood, 1949*b*; for the dog, see Quick, 1932*a*). With the tolylureas (Bray, Lake

& Thorpe, 1949*a*) conjugation may occur through a carboxyl group formed by oxidation of the methyl group or through a new hydroxyl group introduced by nuclear hydroxylation; the extent to which oxidation of the methyl group occurs increases from the *ortho*- to the *para*-isomer, whereas the extent of hydroxylation does the reverse. It was, therefore, of interest to see whether these gradations were a reflexion of the magnitude of the velocity constants of the reactions involved.

Previous determinations of velocity constants have been made using compounds which are readily absorbed by the rabbit, so that after about 2 hr. after dosage the rate of absorption could be neglected. Several hours, however, are required for the absorption of the tolylureas, so that these compounds have provided an opportunity for studying the effect of slow absorption upon the kinetics of conjugation reactions.

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MATERIALS AND METHODS

Materials. The *N*-tolylureas and toluic acids were purchased. *N*-(4-Hydroxy-2-methylphenyl)urea and *N*-(4-hydroxy-3-methylphenyl)urea were prepared as described by Bray *et al.* (1949*a*) and *p*-ureidobenzoic acid was prepared by the method of Menshutkin (1870).

Diet and dosing. Doe rabbits, weighing 2–3 kg., were maintained on a constant diet of rabbit pellets and water (Bray, Ryman & Thorpe, 1947). The acids were usually administered by stomach tube as solutions of their sodium salts and the other compounds as suspensions in water. For intraperitoneal injection solutions of the sodium salts of the acids were adjusted to pH 6–8 and isotonicity with blood. The rats (males, 100–200 g.) used for both excretion and absorption experiments were maintained on a stock diet of rat cubes and water. Administration of accurate doses of the tolylureas by stomach tube was achieved by using a fine suspension prepared by dissolving the tolylurea (1 g.) in hot glycerol (5 ml.) and pouring the solution with vigorous stirring into a 0.1 % solution of bile salts (28 ml.). The suspension formed was stable and easily pipetted, and its preparation did not cause significant decomposition of the tolylureas.

Plan of experiments. The apparatus and experimental procedure used in the kinetic studies with rabbits were as described by Bray, Thorpe & White (1951). For excretion studies with rats, cylindrical metal cages were placed over wire grids above glass funnels containing a wire spiral to prevent mixing of the urine and faeces. To increase the urine volume during these experiments the rats were given water (2 ml.) every 2 hr.

Absorption experiments. Owing to the large number of animals required, the absorption experiments were carried out with rats, kept separately and starved for 16 hr. before dosing. At appropriate times they were killed by a blow on the head. To prevent mixing of contents, the oesophagus was ligated near the cardiac orifice, the duodenum near the pylorus, and the small intestine near the ileo-caecal junction. The contents of the stomach or the small intestine were washed out with water. (A final washing with 20 ml. hot 96 % (v/v) ethanol did not increase the yields of ureido compounds.) The combined washings were gently heated on a water bath to dissolve ureido compounds and then diluted to 1 l. with water. A sample (2 ml.) of the resulting suspension was taken for determination of ureido compounds. Control experiments, in which rats were killed immediately after dosage, gave total recoveries of tolylureas from stomach and intestinal contents ranging from 89 to 93 %.

Determination of conjugates. Ether-soluble acids and ester glucosiduronic acids were determined as described by Bray, Neale & Thorpe (1946), total glucuronic acid and ethereal sulphate as described by Bray *et al.* (1952*b*) and glycine conjugates by the method of Bray, Clowes, Thorpe, White & Wood (1952*a*).

Determination of ureido compounds

Orekhovich & Tustanovsky (1949) reported that a purple colour was produced when a mixture of tryptophan and citrulline (1:3) was heated with diacetyl monoxime in the presence of concentrated acids. The intensity of the colour varied with the tryptophan:citrulline ratio. It has been found that tolylureas can replace citrulline in this reaction and that the intensity of the pink colour formed is pro-

portional to the tolylurea concentration if the tryptophan: tolylurea ratio is not less than 3:1. The interference with the reaction by urea can be effectively eliminated by treatment of the urine with urease.

The following reagents were required: DL-tryptophan (10 mg.) dissolved in water (10 ml.) with the addition of a few drops of phosphoric acid; phosphoric acid (A.R.), sp.gr. 1.75; diacetyl monoxime, 5 % (w/v) solution in 96 % (v/v) ethanol; sodium nitrite, 0.18 % (w/v) solution in water; phosphoric-nitrous acid mixture, sodium nitrite solution (5 ml.) and phosphoric acid (95 ml.) mixed immediately before use. Urease solution was prepared by suspending ground urease tablets (British Drug Houses Ltd.) in 0.1 M sodium phosphate buffer pH 7.0 (45 tablets/100 ml.); after 10 min. the suspension was centrifuged and the supernatant retained for use. Standard solutions of ureido compounds were prepared by dissolving tolylurea (100 mg.), or equivalent amount of other ureido compounds, in 96 % ethanol (10 ml.) and diluting 1 ml. of this solution to 200 ml. with diluted (1:10) rabbit urine or with water as appropriate. This gave a fine suspension and was prepared immediately before use.

Procedure. The solution under examination is diluted to contain 0–50 µg./ml. ureido compound, and a 2 ml. sample of the diluted solution is used. If the solution contains urine, urease solution (2 ml.) is added and the tube incubated for 4.5 hr. at 38°. Phosphoric acid (3 ml.) is then added, followed by tryptophan solution (0.3 ml.) and diacetyl monoxime solution (0.5 ml.). After being shaken vigorously, the tube is heated in a boiling-water bath for 10 min. using a 'cold finger' condenser to reduce evaporation. To the cooled solution phosphoric-nitrous acid mixture (4 ml.) is added and immediately mixed. After 1 min. sodium nitrite solution (0.25 ml.) is added and the mixture again shaken. The colour is measured in a Spekker photoelectric absorptiometer using an Ilford yellow-green filter 605 (max. transmission at 550 mµ.). A calibration curve is constructed from 2 ml. samples of diluted solutions of the ureido compound containing 0–50 µg./ml. A drum reading of 0.1 corresponds to approximately 19 µg. ureido compound for all the compounds studied. Results of a typical recovery experiment using *o*-tolylurea are shown in Table 1. Similar recoveries were obtained with *m*- and *p*-tolylurea, *N*-(4-hydroxy-2-methylphenyl)urea, *N*-(4-hydroxy-3-methylphenyl)urea and *p*-ureidobenzoic acid.

Table 1. *Determination of o-tolylurea added to rabbit urine*

For procedure see text. The *o*-tolylurea in ethanol was added to urine diluted with water (1:10).

Amount added to diluted urine (µg./ml.)	Amount found (µg./ml.)	Percentage recovered
15	15	100
20	21	105
25	24	96
30	29	97
35	35	100
40	39	98
45	43	96
50	48	96
Mean and s.d.		98.5 ± 3

RESULTS

N-(4-Hydroxy-2-methylphenyl)urea and
N-(4-hydroxy-3-methylphenyl)urea

The kinetics of conjugation of these phenols with sulphuric and glucuronic acids in the rabbit were essentially similar to those of other phenols (Bray *et al.* 1952*c, d*), except that a considerable part of the dose (about 40%) was excreted unchanged. At dose levels of 0.5 g./kg. the excretion curves for glucosiduronic acids of both compounds were exponential. In one experiment with *N*-(4-hydroxy-2-methylphenyl)urea, ethereal sulphate was excreted at a constant rate and in another exponentially. This suggests that the dose level used was near the 'critical' dose level (Bray *et al.*

1952*e*) for this compound. In two experiments with *N*-(4-hydroxy-3-methylphenyl)urea the excretion curves for ethereal sulphate were exponential. The first-order velocity constants for glucosiduronic acid and ethereal sulphate formation (k_g and k_s , respectively) calculated from the results of these experiments are given in Table 2. Fig. 1 shows the fit of experimentally determined points with the theoretical curves corresponding to the velocity constants deduced for the two conjugation processes in one of these experiments. The nearly constant g/s quotient (g and s represent the amounts of the phenol excreted as glucosiduronic acid and ethereal sulphate in a given time) is in accord with a system of two simultaneous conjugation reactions following kinetics of the first order.

Table 2. Mean velocity constants for formation of glucosiduronic acid (k_g), conjugation with glycine (k_h), nuclear hydroxylation ($k_{h, OH}$), oxidation of methyl group ($k_{h, COOH}$) and conjugation with sulphuric acid (k_s) for various compounds in the rabbit

Values for k (geometric means) are derived from the arithmetic means of $\log_{10} 100k$ values. Dose levels were 0.2, 0.3 and 0.35 g./kg. for the ureidophenols, tolylureas and toluic acids respectively. Glycine 1.4 g./kg. was administered with the toluic acids. P is the probability that the value of $\log_{10} 100k$ for the compounds is the same as that for the reference compound which heads each section of the table.

Compound	No. of expts.	$\log_{10} 100k$		P	k_g (hr. ⁻¹)
		Mean	S.D.		
Formation of ether glucosiduronic acid					
Phenol*	6	1.748	0.1067	—	k_g 0.56
<i>N</i> -(4-Hydroxy-2-methylphenyl)urea	2	1.111	0.0945	<0.05	0.13
<i>N</i> -(4-Hydroxy-3-methylphenyl)urea	2	1.437	0.0549	<0.05	0.27
Conjugation with sulphuric acid					
Phenol†	7	1.543	0.1594	—	k_s 0.35
<i>N</i> -(4-Hydroxy-2-methylphenyl)urea	1	0.477	—	<0.1	0.03
<i>N</i> -(4-Hydroxy-3-methylphenyl)urea	2	0.801	0.2819	<0.1	0.06
Formation of ester glucosiduronic acid					
Benzoic acid‡	4	0.899	0.1450	—	k_g 0.08
<i>o</i> -Toluic acid	3	1.796	0.1340	<0.001	0.63
<i>m</i> -Toluic acid	3	1.258	0.1639	<0.1	0.18
<i>p</i> -Toluic acid	3	0.168	0.1022	<0.05	0.02
Conjugation with glycine					
Benzoic acid‡	—	—	—	—	k_h 2.6
<i>o</i> -Toluic acid	3	1.175	0.1083	—	0.15
<i>m</i> -Toluic acid	3	1.993	0.0762	—	0.96
<i>p</i> -Toluic acid	3	1.492	0.0140	—	0.31
Nuclear hydroxylation					
<i>N</i> -Phenylurea§	3	1.307	0.0877	—	$k_{h, OH}$ 0.20
<i>o</i> -Tolylurea	4	1.021	0.3457	> 0.1	0.11
<i>m</i> -Tolylurea	2	1.469	0.0352	> 0.1	0.30
Oxidation of methyl group					
Toluene‡	4	1.039	0.1179	—	$k_{h, COOH}$ 0.11
<i>m</i> -Tolylurea	2	1.415	0.0748	<0.1	0.26
<i>p</i> -Tolylurea	3	1.597	0.1052	<0.1	0.40

* Bray *et al.* (1952*c*).

† Bray *et al.* (1952*d*).

‡ Bray *et al.* (1951).

§ Bray *et al.* (1952*b*).

Tolylureas

The excretion curves of metabolites of *o*-, *m*- and *p*-tolylureas may conveniently be considered as having three parts. Fig. 2 illustrates a typical experiment with the *para*-isomer. During an initial phase, lasting for some 2 hr. after dosage, the

Fig. 1. Excretion of glucosiduronic acid and ethereal sulphate by the rabbit after the administration of *N*-(4-hydroxy-3-methylphenyl)urea (0.5 g.). The curves are theoretical ones for $k_g = 0.3 \text{ hr.}^{-1}$ and $k_s = 0.1 \text{ hr.}^{-1}$ and the points (●, glucuronide; ■, ethereal sulphate) are those obtained by experiment. The lower curve, ○—○, shows the quotient of the amounts of phenol excreted as glucosiduronic acid, *g*, and ethereal sulphate, *s*.

Fig. 2. Excretion of metabolites by the rabbit after administration of *p*-tolylurea (0.5 g.). The exponential curve is the theoretical one for $k = 0.37 \text{ hr.}^{-1}$. The points are those obtained by experiment.

slope increased with time to reach a maximum. This was maintained until about 7 hr. after dosage and constitutes a second, rectilinear phase. This was succeeded by an exponential phase, the rate of excretion decreasing proportionally with body level. Thus two velocity constants may be derived from these curves, a zero-order constant from the rectilinear portion and a first-order constant from the exponential part. Since the primary interest of this study was the metabolism of these compounds rather than their absorption, and the first-order velocity constants are an acceptable index of these processes (p. 9), these constants were derived from the last part of the curve and are recorded in Table 2. The nature of the velocity constants which can be derived from the excretion curves of the three tolylureas differ. *o*-Tolylurea is only hydroxylated since oxidation of the methyl group to carboxyl is negligible, so that the only constant to be evaluated is $k_{b,OH}$, the first-order velocity constant for nuclear hydroxylation. With *m*-tolylurea both hydroxylation and oxidation of the methyl group occur, so that values can be obtained for $k_{b,OH}$ and for $k_{b,COOH}$, the first-order velocity constant for the oxidation of the methyl group to carboxyl. *p*-Tolylurea is not appreciably hydroxylated and only $k_{b,COOH}$ can be evaluated.

The derivation of values for $k_{b,COOH}$ presents no difficulties, since the ureido acids formed from *m*- and *p*-tolylureas are rapidly excreted largely unconjugated. The values of $k_{b,OH}$ are, however, of the same order of magnitude as those of $k_g + k_s$, so that values for $k_{b,OH}$ deduced directly from excretion curves are not precise. In a previous investigation (Bray *et al.* 1952*b*), where the relative values of the velocity constants for hydroxylation and conjugation for certain compounds were similar to those found in the present study, it was shown that application of the complete differential equations for the consecutive metabolic processes gave values for velocity constants which were not greatly different from those calculated directly from the rate of excretion. The values for $k_{b,OH}$ recorded in Table 2 were obtained directly from excretion curves.

Toluc acids

Glycine was administered about 1 hr. before the dose of these compounds to ensure a sufficiency of this conjugating moiety (cf. Bray *et al.* 1951). The excretion curves for the *ortho*-, *meta*- and *para*-isomers were exponential, and the appropriate values for k_e and k_h , the first-order velocity constant for conjugation with glycine, are recorded in Table 2. The values for *p*-toluic acid are of the same order as those previously found (0.06 and 0.4 hr.^{-1} , Bray *et al.* 1955).

Absorption of ureido compounds

The results of absorption experiments on rats are summarized in Fig. 3. The dose levels used in these experiments were comparable with those used in experiments with rabbits. It can be seen that absorption of the ureidophenols and *p*-ureidobenzoic acid is almost complete in 3 hr. while the absorption of the tolylureas is relatively slow.

Fig. 3. The absorption of ureido compounds by the rat. Points represent the average results from 3 to 6 rats. Doses were: tolylureas, *o*-, 50 mg.; *m*-, 44 mg.; *p*-, 37 mg.; *N*-(4-hydroxy-2-methylphenyl)urea, 30 mg.; *N*-(4-hydroxy-3-methylphenyl)urea, 35 mg.; *p*-ureidobenzoic acid, 39 mg. The compounds are represented by □, ○, △, ■, ●, ▲, respectively.

The amounts of ureido compounds found in the large intestine during absorption were negligible. In the case of the tolylureas most of the unabsorbed material was in the stomach, which suggests that the emptying of the stomach may be a determining factor in the absorption of these compounds.

DISCUSSION

In the previous kinetic studies (Bray *et al.* 1951; 1952*b, c, d, e*; 1955) the compounds administered were rapidly absorbed, and the time taken by absorption was small compared with the time occupied by metabolism and excretion of metabolites. Under these conditions it was shown that the effect of absorption time on the determination of values of velocity constants was very small (Bray *et al.* 1951). In contrast to these compounds, the tolylureas are absorbed so slowly that it is necessary to consider the effect of absorption on excretion curves. Following the administration of

tolylureas to a rabbit, there is a period during which the rate of excretion of metabolites is constant. Since it is likely, under the conditions of these experiments, that the rates of metabolic processes undergone by these compounds are proportional to their body levels it might be expected that the body levels would be constant during the period over which the excretion curve is rectilinear. When the tolylureas were fed to rats it was, in fact, found that the experimentally determined

Fig. 4. The absorption and excretion of *p*-tolylurea (dose 0.26 g./kg.) administered to rats. Each point on the absorption curve, □—□, represents the average result from three rats. The points on the excretion curve, ○—○, were obtained from the urine of one rat; water (2 ml.) was given every 2 hr. The curve without experimental points represents the body level (amount absorbed less amount excreted) and is derived from the other two curves.

body level, i.e. the amount fed less the amounts unabsorbed and excreted, remained almost constant. This is illustrated by an experiment with *p*-tolylurea shown in Fig. 4. This should be compared with Fig. 5, which shows the corresponding curves from an experiment with a rapidly absorbed compound, *N*-(4-hydroxy-3-methylphenyl)urea, where the body level rises sharply and then soon begins to fall exponentially. The rectilinear phase of the tolylurea excretion curves is followed by a similar fall in body level corresponding to the exponential portion of the excretion curve (e.g. see Table 3). There are thus two ways in which values for the velocity constants for the metabolic processes undergone by the tolylureas may be derived. The first is the usual method, applying the 'logarithm' or 'tangent' methods to the terminal exponential portion of the excretion curve and the second is from the quotient of rate of excretion of metabolites and body level over the rectilinear portion of the curve. The application of the two

methods is illustrated by Table 3. The value for the 2-7 hr. period was calculated from the average rate of excretion and body level over that period, and the remaining five values from instantaneous body levels and rates. Application of the 'log' or 'tangent' method to the portion of the curve between 8 and 12 hr. gives a mean value of 0.31 hr.^{-1} , which is in good agreement with 0.29 hr.^{-1} obtained over the 2-7 hr. period, and is of the same order as that obtained for the rabbit (0.39 hr.^{-1} , Table 2).

Fig. 5. The absorption and excretion of *N*-(4-hydroxy-3-methylphenyl)urea (dose 0.21 g./kg.) administered to rats. Each point on the absorption curve, $\square$ — $\square$, represents the average result from three rats. The excretion curve, $\circ$ — $\circ$, was obtained from the urine of one rat; water (2 ml.) was given every 2 hr. The curve without experimental points represents the body level and is derived from the other two curves.

Table 3. Velocity constants for oxidation of the methyl group of *p*-tolylurea by the rat determined at various times after dosage

The value for the 2-7 hr. period corresponds to the rectilinear part of the excretion curve and the other values correspond to the subsequent exponential curve.

Time after dosage (hr.)	Body level (% of dose)	Excretion rate (% of dose/hr.)	k_b (hr.^{-1})
2-7	24	7	0.29
8	19	5	0.26
9	14	4.3	0.31
10	10	2.85	0.29
11	7	2.25	0.32
12	5	1.75	0.35

This investigation has provided an example of another factor which may give rise to linear phases in excretion curves in kinetic studies. Previously the linear portions observed in excretion curves were the result of a deficiency of a conjugating moiety, e.g. glycine (Bray *et al.* 1951, 1955) or sulphate (Bray *et al.* 1952*d*). In these cases absorption of administered compound was rapid and usually, if the conjugating moiety or its precursor was administered along with the dose, an exponential excretion curve was obtained, although satisfactory exponential excretion curves have not yet been obtained from unsubstituted benzoic acid. When a linear excretion curve is the result of slow absorption it cannot be changed into an exponential curve by administration of conjugating moiety. Suitable analysis of the curve can, however, lead to the derivation of satisfactory values for velocity constants for metabolic processes. It is not yet known whether this procedure is generally applicable, since the absorption patterns of different compounds may vary widely.

It was previously noted (Bray *et al.* 1952*c*) that the specific rate constants for the formation of glucosiduronic acids, k_g , from monosubstituted phenols did not differ significantly from that of phenol itself if the weight of the substituent radical did not exceed that of chlorine. For phenols with radical weights greater than this, k_g values were significantly lower than that of phenol. Although the two phenols included in the present study are not monosubstituted phenols, the radical weight of the ureido group, 59, is much greater than that of chlorine and the values of k_g for both are significantly smaller than that for phenol (Table 2). The value for *N*-(4-hydroxy-2-methylphenyl)urea (0.13 hr.^{-1}) is of the same order as that for *N*-(*p*-hydroxyphenyl)urea (0.11 hr.^{-1} , Bray *et al.* 1952*c*), while that for *N*-(4-hydroxy-3-methylphenyl)urea is greater (0.27 hr.^{-1}). The values for k_e (0.03 and 0.07 hr.^{-1}) are the smallest which we have observed, although of the same order as that of *N*-(*p*-hydroxyphenyl)urea (0.11 hr.^{-1} , Bray *et al.* 1952*d*).

One purpose of this investigation was to see whether the gradations in metabolic fate of the two series of isomers were reflected in the values of the appropriate rate constants. The relevant values are shown in Table 4. For reasons given previously (Bray *et al.* 1952*e*) a direct correlation between the total amount of each metabolite excreted and the corresponding velocity constant would not be expected. In the toluic acids the conjugations with glucuronic acid and glycine both follow first-order kinetics so that the quotient, A/B , of the total amounts of the two conjugates should be the same as the quotient of the respective velocity constants, k_g/k_h . It can be seen from Table 4 that these quotients are, in fact, in close agreement, that the

gradation in the *ortho*-, *meta*- and *para*-isomers is determined chiefly by variations in k_g and that the irregular gradation of k_h has comparatively little effect upon the gradation in the quotient.

In the tolylureas the methods of analysis available were not sufficiently precise for the measurement of amounts of metabolites excreted to an extent of less than 1% of the dose and for the consequent evaluation of the velocity constants. Since neither oxidation of the methyl group of

of the conjugation of *N*-(4-hydroxy-2-methylphenyl)urea and *N*-(4-hydroxy-3-methylphenyl)urea with glucuronic and sulphuric acids in the rabbit have been studied. Values are given for the appropriate velocity constants.

2. The effect of the slow absorption of the tolylureas on the form of the excretion curves obtained after their administration is considered. The derivation of values for velocity constants from such excretion curves is discussed.

Table 4. Relation between mean velocity constant and percentage of dose excreted for reactions of the *ortho*-, *meta*- and *para*-isomers of toluic acids and tolylureas in the rabbit

Isomer	Conjugation as ester glucosiduronic acid		Toluic acids		A/B	k_g/k_h
	(% of dose) A	k_g (hr. ⁻¹)	Conjugation with glycine			
			(% of dose) B	k_h (hr. ⁻¹)		
<i>Ortho</i>	70	0.63	17	0.15	4.12	4.20
<i>Meta</i>	13	0.18	77	0.96	0.17	0.19
<i>Para</i>	4	0.02	62	0.31	0.065	0.065

Isomer	Oxidation of methyl group		Tolylureas		C/D	$k_b, \text{COOH}/k_b, \text{OH}$
	Excretion as free ureido-benzoic acid and ester glucosiduronic acid (% of dose) C	k_b, COOH (hr. ⁻¹)	Nuclear hydroxylation			
			Excretion as phenolic conjugates of glucuronic and sulphuric acids (% of dose) D	k_b, OH (hr. ⁻¹)		
<i>Ortho</i>	0	0	40	0.11	0	0
<i>Meta</i>	53	0.26	46	0.30	1.15	0.87
<i>Para</i>	90	0.39	0	0	∞	∞

o-tolylurea nor hydroxylation of *p*-tolylurea could be detected by these methods the values for the quotients of the amounts excreted and of the velocity constants are not finite for these two isomers and the exact values of the velocity constants play little part in determining the metabolic fate of the *ortho* and *para* compounds. The two processes measured both follow first-order kinetics and the values of the quotients C/D and $k_b, \text{COOH}/k_b, \text{OH}$ should be of the same order. That the agreement for the *meta*-isomer is not as close as for the toluic acids is probably due to experimental error in evaluation of the rate constants which in each case required two determinations (ether-soluble acid + ester glucuronide and ether glucuronide + ethereal sulphate) and depend on four base-line values.

SUMMARY

1. The kinetics of the conjugation of the toluic acids with glycine and glucuronic acids, of the oxidation and hydroxylation of the tolylureas, and

3. The gradation in the relative amounts of metabolites of *ortho*-, *meta*- and *para*-isomers excreted is discussed in relation to the velocity constants of the reactions involved.

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The Chemistry of Connective Tissues

2. SOLUBLE PROTEINS DERIVED FROM PARTIAL HYDROLYSIS OF ELASTIN*

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By contrast with other components of connective tissue, relatively few chemical studies on the structure of elastin have been reported. This may be due to the inherent difficulty of studying a protein which is characteristically insoluble and resistant to the attack of mild reagents. As in the case of other fibrous proteins, the only feasible approach to a chemical study of structure appears to be by a process of stepwise degradation, and the aim of this work was to prepare the ground for such a study by an examination of the products of mild acid hydrolysis.

During an investigation of the early stages of hydrolysis of various mucoids and connective-tissue proteins, it was observed that, on treatment with dilute acetic acid or oxalic acid at 100°, aspartic acid was in each case the first free amino acid to appear, followed at a later stage by glutamic acid (Partridge & Davis, 1950). Since elastin has an unusually low content of the dicarboxylic amino acids (Stein & Miller, 1938; Neuman, 1949) and has a low content of serine and threonine, which also give rise to easily hydrolysable peptide bonds (Desnuelle & Bonjour, 1952), it was thought that the first products of hydrolytic cleavage might consist of a mixture of soluble degradation products of high molecular weight. It was later observed (Adair, Davis & Partridge, 1951) that six successive extractions, each with 0.25*M* oxalic acid at 100° for 1 hr., were sufficient to bring dried and powdered elastin from *ligamentum nuchae* of cattle completely into solution.

If a study of the degradation products is to give information which is relevant to the elastin fibre as it exists in elastic tissue, it is important to know if the fibre is itself homogeneous. Morphologically,

the elastin fibre has for many years been recognized as an entity, and autoclaved preparations of elastin, particularly those from *ligamentum nuchae*, are usually considered to be homogeneous chemically. The work reported in this series of papers supports the view that purified preparations of elastin are chemically homogeneous, although there seems reason to suppose that there may be structural heterogeneity at macromolecular level.

The protein fibre as it exists in elastic tissue is closely associated with collagen. Thus Astbury (1940) observed a typical collagen X-ray diffraction pattern in stretched *ligamentum nuchae*, but this changed to amorphous rings when collagen was removed by autoclaving.

Wolpers (1944) treated *ligamentum nuchae* with pepsin for 24 hr. in order to remove collagen and obtained fibres of a size suitable for study with the electron microscope. The preparation was revealed as large branching amorphous fibres ranging in width from 2500 Å to 200 Å. The fibres appeared to have no axial periodicity.

McCartney (1914) showed that after removing collagen from *ligamentum nuchae* by peptic digestion the fibres contract linearly with increase in temperature and return to their original length on cooling. Jordan Lloyd & Garrod (1948) compared the thermoelastic behaviour of elastin to that of rubber and deduced that in the unstretched fibre the peptide chains are highly contorted and are cross-linked at a few points only. They found that if the fibre is dried in the stretched condition it remains in the extended state after release of the tension. This was explained as being due to the polar properties of the peptide bonds, which introduce further cross-links when water is removed. Imbibition of water is necessary to keep the chains apart, and thus to permit thermal

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