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Studies in Detoxication

24. THE METABOLISM OF *p*-PHENETIDINE (*p*-ETHOXYANILINE) WITH SOME OBSERVATIONS ON THE ANISIDINES (METHOXYANILINES)

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This study of the fate of alkoxyanilines in the rabbit was pursued because we wanted to know the effect of alkoxy groups on the biological acetylation of the amino group in compounds of the type $R \cdot C_6H_4NH_2$. The study became of further interest when it was found that administration of *p*-phenetidine and the *o*-, *m*- and *p*-anisidines resulted in the excretion of reducing urines as in the case of aniline (Smith & Williams, 1949b).

Little previous work has been done on *p*-phenetidine; Edlerson (1900) showed that, in man, it gave rise to conjugated *p*-aminophenol and Elson, Goulden & Warren (1946) suggested that it behaved similarly in the rat.

EXPERIMENTAL

Methods. The quantitative estimations of the excretion of glucuronic acid, ethereal sulphate, and diazotizable amino groups in the urine of rabbits receiving *p*-phenetidine hydrochloride orally are quoted in the preceding paper (Smith & Williams, 1949b).

Phenetidine was fed as the hydrochloride, m.p. 234°.

The isolation of phenetidine metabolites(1) *The nature of phenetidine urine*

After feeding phenetidine in doses of 0.2–0.5 g./kg., rabbits excreted a brown urine (pH c. 8) similar in appearance to aniline urine. It reduced Benedict's reagent and gave a very rapid naphthoresorcinol reaction. It gave a permanganate colour with $FeCl_3$ and a positive diazo reaction.

The urine was optically active. The urine from two rabbits, each of which had received 2 g. of phenetidine

hydrochloride, was estimated to contain 4.7 g. of extra glucuronic acid. A 10 ml. portion of this urine was clarified with dialyzed iron and made up to 20 ml. In a 2 dm. tube α_D was -0.42° , whilst normal rabbit urine treated in the same way has no detectable rotation. If the glucuronide in the urine were that of either *p*-aminophenol or *p*-acetamidophenol, the expected α_D would be $c. -1.3^\circ$. Thus it follows that the glucuronides from phenetidine cannot be entirely *p*-aminophenol derivatives.

(2) *Isolation of unchanged phenetidine*

The filtrate from the preparation of the basic lead acetate fraction of phenetidine urine (see p. 251) was made alkaline with KOH, filtered and continuously extracted with ether for 4 hr. The bases in the ether were extracted with 2N-HCl. This solution was made alkaline and extracted in a funnel with ether. This ethereal solution was dried over NaOH for 24 hr., filtered, and saturated with dry HCl gas. The crystalline precipitate of phenetidine hydrochloride (m.p. and mixed m.p. 234°) was filtered off. The yields of unchanged phenetidine in these experiments were 1.5% of the dose at a level of 0.5 g./kg. and 2 and 5% at 0.8 g./kg.

(3) *The ethereal sulphate fraction*

(a) *Isolation of 2-hydroxy-4-ethoxyaniline (hydroxyphenetidine).* The 24 hr. urine (1250 ml.) after feeding 18 g. phenetidine hydrochloride was concentrated at 40° *in vacuo* to 250 ml., acidified with dilute HCl, saturated with $(NH_4)_2SO_4$ and extracted with 2 × 250 ml. acetone. The acetone extract was made alkaline with solid K_2CO_3 , concentrated *in vacuo* to 100 ml., treated with 1 l. dry acetone, filtered and concentrated to 50 ml. The concentrate was now extracted five times with an equal volume of ether to remove free phenetidine (recovered as the hydrochloride, m.p. 230°). (Found: C, 55.1; H, 6.9; N, 7.6. Calc. for

$C_8H_{11}ON \cdot HCl$: C, 55.3; H, 7.0; N, 8.0%.) Further concentration of the extracted solution caused the separation of urea and some inorganic material which were removed by shaking with 95% ethanol and filtering. The filtrate, now containing largely ethereal sulphates with some glucuronide, was treated with 300 ml. absolute ethanol which threw out the glucuronide as a non-reducing flocculent precipitate (600 mg.) which we were unable to identify. The ethanolic solution was now reduced *in vacuo* to a syrup (30 ml.), which at this stage gave negative tests for glucuronic acid, but positive ethereal sulphate and diazo tests. However, we were unable to isolate the sulphate or a derivative in crystalline form. Therefore 10 ml. of the syrup were dried *in vacuo*, and the residue boiled for 5 min. with 5 ml. conc. HCl and 20 ml. of 5% aqueous $BaCl_2$. After cooling, the $BaSO_4$ was filtered off and the filtrate treated with a little $Na_2S_2O_4$ which removed some of the dark brown colour. Then it was made alkaline with K_2CO_3 , stirred with 5 ml. acetic anhydride for 10 min., and extracted with 20 ml. chloroform. The extract was dried over $CaCl_2$ and allowed to evaporate at room temperature. A sticky mass of crystals separated which became readily filterable on addition of ether. Recrystallization from benzene gave rectangular plates (170 mg., 2.5% of the dose) of 2-acetoxy-4-ethoxyacetanilide, m.p. and mixed m.p. 127–129°. (Found: OC_2H_5 , 19.8. Calc. for $C_{12}H_{15}O_4N$: OC_2H_5 , 19.0%.)

(b) *Synthesis of 2-acetoxy-4-ethoxyacetanilide*. 2-Hydroxy-4-ethoxyaniline hydrochloride, prepared according to Henrich & Birkner (1913), yielded with aqueous Na_2CO_3 and acetic anhydride 2-acetoxy-4-ethoxyacetanilide, m.p. 127–129° after recrystallization from benzene.

A more convenient method of synthesis starts from 4-nitrosoresorcinol 1-ethyl ether, which was prepared according to the method of Henrich & Rhodius (1902) for the methyl ether. 4-Nitrosoresorcinol 1-ethyl ether (0.5 g.) was reduced with 2 g. of Sn in 5 ml. conc. HCl. The reduction mixture was evaporated on the water bath to remove excess HCl, neutralized with $NaHCO_3$ and extracted with ether to remove 2-hydroxy-4-ethoxyaniline. The extract was treated with an excess (10 ml.) of acetic anhydride and evaporated *in vacuo* to a small volume. The residue was mixed with an excess of Na_2CO_3 solution, and the whole shaken until the oil, which had separated, crystallized. The 2-acetoxy-4-ethoxyacetanilide (0.27 g.) was dried and recrystallized from benzene. It formed rectangular plates, m.p. 127–129°. (Found: C, 60.5; H, 6.05; N, 6.1; OC_2H_5 , 19.4. $C_{12}H_{15}O_4N$ requires C, 60.7; H, 6.4; N, 5.9; OC_2H_5 , 19.0%.)

(4) The glucuronide fraction

(a) *Detection of a glucuronide of p-aminophenol*. The basic lead acetate precipitate of a 24 hr. urine (340 ml.) from two rabbits, each of which had received 2 g. of *p*-phenetidine hydrochloride, was prepared in the usual manner. It was suspended in water and Pb removed with H_2S . The lead-free filtrate was concentrated *in vacuo* to a brownish gum (5 g.) which had properties similar to that obtained from aniline urine. Part of this gum (4.6 g.) was treated with 150 ml. of ethereal diazomethane (from 5 g. nitroso-methylurea). The ether was removed and the neutral tarry residue dissolved in a mixture of 20 ml. pyridine and 20 ml. acetic anhydride. After 18 hr. at room temperature the mixture was diluted with 100 ml. water and extracted with 3 × 20 ml. chloroform. After washing with dilute acid and alkali and drying over $CaCl_2$, the chloroform extract was

evaporated, leaving a partially crystalline gum (0.94 g.). This product was dissolved in hot ethanol and on cooling to 0°, 150 mg. (1.5% of the dose) of *p*-acetamidophenyltri-acetylglucuronide methyl ester (m.p. and mixed m.p. 205–207°); $[\alpha]_D^{15} - 23.5^\circ$ (c, 6.1 in chloroform) separated (Smith & Williams, 1948, 1949b).

Isolation of this compound showed that the glucuronide fraction contained either *p*-aminophenylglucuronide or its *N*-acetyl derivative or both.

(b) *Isolation of p-acetamidophenylglucuronide*. The glucuronide fraction was prepared by lead acetate treatment from the 24 hr. urine of 6 rabbits which had collectively received 12 g. phenetidine hydrochloride. This fraction was concentrated so that 50 ml. of an aqueous syrup was obtained. This was now diluted with 400 ml. absolute ethanol which was added with shaking. A gum was thrown out and the ethanol (A) removed by decanting. The gum was dissolved in 10 ml. water and on treating this with 5 g. *p*-toluidine in 5 ml. ethanol and cooling to 0° overnight, 8 g. of the *p*-toluidine-ammonium glucuronate complex (m.p. 125°) crystallized and was filtered off.

The ethanolic solution A was now evaporated to 20 ml. and again diluted to 250 ml. with ethanol to throw out a further portion of insoluble gum. Again the ethanol was decanted and the process repeated. The insoluble gum obtained yielded a further 1 g. of *p*-toluidine-ammonium glucuronate complex. The mother liquor, after removal of the insoluble gum, was now taken to dryness *in vacuo*. The residual gum (5 g.) gave no diazo reaction until hydrolyzed: 4.7 g. of it were dissolved in 50 ml. 95% ethanol, and 3 ml. benzylamine were added. The mixture was diluted to 500 ml. with ethyl acetate. From this solution there was obtained 2 g. (6.4% of the dose) of the benzylamine salt, of *p*-acetamidophenylglucuronide, m.p. and mixed m.p. 195–198° (after recrystallization from 95% ethanol), $[\alpha]_D^{25} - 59^\circ$ (c, 7.8 in water). (Found: N, 6.5. Calc. for $C_{21}H_{26}O_8N_2 \cdot H_2O$: N, 6.2%.)

(c) *Attempted isolation of p-aminophenylglucuronide*. *p*-Aminophenylglucuronide was isolated from aniline urine from the mercuric acetate fraction (see Smith & Williams, 1949b, preceding paper). An attempt was made to isolate it from phenetidine urine by a similar procedure. We thought that it might occur in phenetidine urine in small amounts, but its isolation as such or as a benzylamine salt was not achieved.

(d) *Spectroscopic observations on the glucuronide fraction*. As in the case of aniline, the absorption spectrum of the glucuronide fraction of *p*-phenetidine urine can be accounted for by the presence of relatively small amounts of aminophenylglucuronides. An aqueous solution (400 ml.) of the glucuronide fraction, prepared, after feeding 8.75 g. phenetidine, as in section 4 (a) above, was estimated (naphthoresorcinol method) to contain 7.1 g. of glucuronic acid. It was made faintly alkaline with ammonia, diluted 100 times with water and its ultraviolet absorption measured in a Hilger E3 spectroscope. It had a maximum extinction ($E_{0.3 \text{ cm.}}$) at 240 mμ. of 1.2. The form of the absorption curve closely resembled that of *p*-acetamidophenylglucuronide (Smith & Williams, 1948) and the presence of *p*-aminophenylglucuronide was not indicated. If it be accepted that the absorption was due to *p*-acetamidophenylglucuronide, then it can be calculated from the value of $E_{0.3 \text{ cm.}}$ that 4.9 g. of it is present, i.e. 2.9 g. or 41% of the total glucuronic acid.

(5) *The isolation of free glucuronic acid from the urine of rabbits receiving alkoxyanilines orally*

(a) *p*-Phenetidine. The glucuronide gum from the urine of twelve rabbits which had collectively received 24 g. of *p*-phenetidine hydrochloride was prepared (see section 4 (a)). From this the *p*-toluidine-ammonium glucuronate complex was prepared as described in the preceding paper on aniline (Smith & Williams, 1949b). The yield was 34 g. or 58% of the dose assuming that one molecule causes the excretion of one of glucuronic acid. It was recrystallized (plates) from hot aqueous ethanol. (Found: C, 56.55; H, 7.4; N, 10.7; OC_2H_5 , 0; glucuronic acid, 47.6; *p*-toluidine, 48; H_2O , 4.5. $\text{C}_{20}\text{H}_{29}\text{O}_6\text{N}_3 \cdot \text{H}_2\text{O}$ requires C, 56.45; H, 7.3; N, 9.9; OC_2H_5 , 0; glucuronic acid, 45.6; *p*-toluidine, 50.4; H_2O , 4.2%.) It decomposed at 125–128° and showed $[\alpha]_D^{20} - 60^\circ \xrightarrow{24 \text{ hr.}} +2.5^\circ$ (constant value) (c, 0.5 in 1:3 ethanol-water), $[\alpha]_D^{20} + 18 \rightarrow +11^\circ$ (constant) (c, 5.2 in 0.4N-HCl).

The complex was analyzed for *p*-toluidine by heating on the water bath for 0.5 hr. with 0.5N-HCl, cooling, then diazotizing and coupling with naphthylethylenediamine. The diazo colour was measured in a Spekker absorptiometer and compared with a standard calibration curve constructed with pure *p*-toluidine hydrochloride.

The water content of the complex could not be determined directly by drying to constant weight because it decomposed with loss of toluidine. An approximate estimate of the water content of the sample analyzed above was obtained using Fischer's reagent (pyridine, I_2 and SO_2 ; see Fischer, 1935). As mentioned in the preceding paper, we had reason to believe that this compound formed two hydrates similar to the glucose *p*-toluidides of Irvine & Gilmour (1909). Our experiments suggested that the hydrate containing $1.5\text{H}_2\text{O}$ was formed when crystallization was carried out at 0° (see section 6 (b)) and the one containing H_2O was formed at room temperature or higher. When the sample above was recrystallized at 0° it gave C, 55.9; H, 7.05; N, 9.6. $\text{C}_{20}\text{H}_{29}\text{O}_6 \cdot 1.5\text{H}_2\text{O}$ requires C, 55.3; H, 7.4; N, 9.7%.

When the compound was dissolved in cold 20% NaOH it gave off ammonia (proved by aeration into picric acid solution, to give ammonium picrate, m.p. and mixed m.p. 270° (decomp.)). In the Van Slyke apparatus the compound released N_2 in 0.5 hr. equivalent to 3.2% N; the first formula quoted above requires 3.3% N as NH_4 .

(b) *o*-Anisidine. *o*-Anisidine (3.6 g., b.p. 218–220°) was dissolved in the minimum of dilute HCl and the solution diluted to 40 ml. Two rabbits each received 20 ml. of this solution. It had a slight narcotic effect but the animals were easily roused by handling. One rabbit died 5 days later. A reducing urine was excreted even during the first 2 hr. after feeding. The 24 hr. urine (380 ml.) was brown and darkened further on standing. The colour was discharged by $\text{Na}_2\text{S}_2\text{O}_4$. The urine reduced Fehling's solution, gave a strong red diazo reaction and an intense Tollens test. The glucuronide gum (3 g.) was prepared as for phenetidine urine and, on treatment with *p*-toluidine as before, 1.3 g. (equivalent to 14% of the anisidine fed) of the *p*-toluidine-ammonium glucuronate complex (m.p. 125–128° decomp.) was obtained; $[\alpha]_D^{20} + 18^\circ$ (initial value) (c, 3.9 in 0.4N-HCl). Absorption spectrum: λ_{max} 234 m μ ., ϵ_{max} 16,800 in water. (Found: C, 56.0; H, 7.3; N, 9.6%.)

(c) *p*-Anisidine. Two rabbits were each fed with 2 g. of

p-anisidine (m.p. 57°) in 20 ml. of water containing the minimum amount of dilute HCl. The urine was reducing, and the glucuronide gum (3 g.), prepared as before, yielded 1 g. (equivalent to 9.5% of the dose) of the *p*-toluidine-ammonium glucuronate complex (m.p. 125–128° decomp.), $[\alpha]_D^{20} + 20^\circ$ initial (c, 5.4 in 0.4N-HCl). (Found: C, 56.25; H, 7.1; N, 8.8%.)

(d) *m*-Anisidine. In a similar fashion the toluidine complex was obtained after feeding *m*-anisidine (b.p. 246–247°/720 mm.). It had m.p. 125–128° decomp., $[\alpha]_D^{20} + 19 \rightarrow +15^\circ$ (c, 5 in 0.4N-HCl) and spectral absorption λ_{max} 234 m μ ., ϵ_{max} 16,600 and λ_{max} 285 m μ ., ϵ_{max} 2600 in water.

(6) *Synthesis of p-toluidine-ammonium uronate complexes*

(a) *D*-glucurone. When *p*-toluidine in ethanol is added to an aqueous solution of glucurone containing a little ammonia, no *p*-toluidine complex separates.

(b) *D*-glucuronic acid. *D*-Glucurone (0.5 g.) was converted into barium glucuronate by treatment with 1 equiv. of 0.3N-Ba(OH) $_2$ at room temperature for 3 hr.; 0.5 ml. of saturated $(\text{NH}_4)_2\text{SO}_4$ solution was added and the mixture filtered from BaSO_4 . To the filtrate was added 0.6 g. *p*-toluidine in 5 ml. ethanol and then the mixture was diluted to 50 ml. with ethanol. After 24 hr. the crystals (0.54 g., 45% of theory) of the *p*-toluidine-ammonium glucuronate complex were filtered off and washed with water and ethanol. It was recrystallized from ethanol-water and cooled to 0°. Rectangular plates were obtained, m.p. 125–130° (decomp.), $[\alpha]_D^{20} - 59^\circ \xrightarrow{24 \text{ hr.}} +2^\circ$ (constant value) (c, 0.3 in 1:3 ethanol-water). (Found: C, 55.3; H, 7.4; N, 9.7. $\text{C}_{20}\text{H}_{29}\text{O}_6 \cdot 1.5\text{H}_2\text{O}$ requires 55.3; H, 7.4; N, 9.7%.) Ultraviolet absorption in water: λ_{max} 234 m μ ., ϵ_{max} 16,800 and λ_{max} 285 m μ ., ϵ_{max} 2600. It was insoluble in ethanol and organic solvents and sparingly soluble in water. It reduced Benedict's reagent readily and gave a rapid Tollens test for glucuronic acid. It was identical in all respects with the compound obtained from the alkoxy-aniline urines.

The same complex was obtained from crude barium glucuronate prepared by acid hydrolysis of Turkey gum.

(c) From (–)-Menthyl-*D*-glucuronide. The complex (m.p. 123° decomp.) was obtained in poor yield (7%) from an acid-hydrolyzed solution of menthylglucuronide. (Found: C, 56.3; H, 7.2; N, 9.4% ($[\alpha]_D^{20} + 27 \rightarrow +15^\circ$) (c, 2.7 in 0.4N-HCl).)

(d) *The complex from D-galacturonic acid.* When an aqueous solution of *D*-galacturonic acid is mixed with an equal volume of an ethanolic solution of *p*-toluidine, no apparent change takes place. But as soon as the mixture is treated with a few drops of ammonia solution (sp.gr. 0.88) it crystallizes completely. The *p*-toluidine-ammonium *D*-galacturonate complex, obtained in excellent yield, forms plates, m.p. 135–139° (decomp.) and $[\alpha]_D^{20} - 31 \rightarrow +5^\circ$ (constant value after 24 hr.) (c, 1.2 in 50% aqueous ethanol). Its absorption spectrum is similar to that of the glucuronic acid analogue; in water it shows λ_{max} 232 m μ ., ϵ_{max} 16,600; λ_{max} 285 m μ ., ϵ_{max} 2850. (Found: C, 56.5; H, 7.3; N, 9.5. $\text{C}_{20}\text{H}_{29}\text{O}_6\text{N}_3 \cdot \text{H}_2\text{O}$ requires C, 56.45; H, 7.3; N, 9.9%.) This was slightly more soluble than the glucuronic acid complex.

DISCUSSION

It is clear that the metabolism of *p*-phenetidine is a complex problem which requires further investigation. So far we have proved that it undergoes (a) de-ethylation, (b) acetylation, and (c) oxidation without de-ethylation.

The occurrence of de-ethylation and deacetylation was proved by the isolation of *p*-acetamidophenylglucuronide, which could be formed in two ways as follows:

The first of these mechanisms appears to be the most likely, for if *p*-aminophenol were formed we would have expected to isolate its glucuronide, but we did not. For phenetidine the difference between the free and total diazotizable amino groups amounted to 22% (see Table 1 of the preceding paper, Smith & Williams, 1949*b*). This figure, together with a consideration of the spectrum of the glucuronide gum, suggests that about one quarter of the phenetidine fed is excreted as compounds containing the acetamido group.

It was shown (Smith & Williams, 1949*a*) that the de-ethylation of phenacetin *in vivo* was virtually complete, the main excretion products being *O*-conjugates of *p*-acetamidophenol. With phenetidine, however, there is only a partial de-ethylation, for we isolated from the urine 2-hydroxy-4-ethoxyaniline in which the original ethoxyl group of phenetidine is intact. It appears, therefore, that breaking of the ether linkage *in vivo* takes place more readily if the *p*-amino group is acetylated. The isolation of 2-hydroxy-4-ethoxyaniline is also of interest in relation to the orientation of the hydroxyl group entering the phenetidine molecule. Both the NH_2 and OC_2H_5 groups are *o-p*-directing (ring activating), but the oxidation takes place *o*- to the amino group. The amino group is known to be chemically more strongly directing than the alkoxy group (Fieser & Fieser, 1944) and the isolation of 2-hydroxy-4-ethoxyaniline fits in with this view.

Rabbits receiving phenetidine excrete ethereal sulphates corresponding to 30% of the dose. Hydrolysis of the sulphate fraction yielded a reddish tar consisting largely of phenolic substances, from which 2-hydroxy-4-ethoxyaniline was isolated in yields of 8–9% of the ethereal sulphate fraction (i.e. 2.5% of the dose). We failed to detect *p*-aminophenol in this fraction, but there were suggestions that 4-aminoresorcinol was present. It appears, therefore, that the main phenol in the sulphate fraction is 2-hydroxy-4-ethoxyaniline.

The exact nature of the glucuronide fraction of phenetidine urine has not been solved, although it

is clear from this work that it presents, as in the case of aniline, a new aspect of the metabolism of aromatic amines. On measuring the 'extra' glucuronic acid excretion it was found that more than one molecule of glucuronic acid was excreted/molecule of phenetidine fed. The average glucuronide output was 120% of the dose. This high figure could mean that a metabolite carrying two glucuronic acid molecules is excreted. Alternatively, it may mean that phenetidine stimulates the excretion of free glucuronic acid. The *p*-toluidine complex

of glucuronic acid was isolated in large amounts, equivalent to 58% of the phenetidine fed, and we believe that a considerable proportion of phenetidine is excreted as a labile glucuronide. A minor component of the glucuronide fraction is *p*-acetamidophenylglucuronide, which was isolated. Although

Fig. 1. Ultraviolet absorption spectra of *p*-toluidine-ammonium uronate complexes. — *A*, *p*-Toluidine-ammonium *D*-glucuronate complex from aniline urine in water: λ_{max} 234 μ , ϵ_{max} 16,900 and λ_{max} 285 μ , ϵ_{max} 2700; *B*, same as *A* but in *N*-HCl: λ_{max} 261, ϵ_{max} 400; ----- synthetic *p*-toluidine-ammonium *D*-glucuronate complex (prepared from *D*-glucuronic acid) in water: λ_{max} 234 μ , ϵ_{max} 16,800 and λ_{max} 285 μ , ϵ_{max} 2600; -.-.- *p*-toluidine-ammonium *D*-galacturonate complex (prepared from *D*-galacturonic acid) in water: λ_{max} 232 μ , ϵ_{max} 16,600 and λ_{max} 285 μ , ϵ_{max} 2850.

we isolated *p*-aminophenylglucuronide from aniline urine, it was not found in phenetidine urine, a fact which is supported by the very low amount (3% of the dose) of free diazotizable amino groups found in phenetidine urine.

The evidence presented here on the fate of phenetidine in the rabbit does not allow us to make very definite conclusions and we suggest the above scheme very tentatively.

The *p*-toluidine-glucuronate complex and the labile glucuronides derived from aromatic amines. The *p*-toluidine-ammonium glucuronate complex has been isolated from *p*-phenetidine (58 % of the dose), aniline (21 %), *o*-anisidine (14 %), *m*-anisidine (12 %) and *p*-anisidine (9.5 %) urines. Only traces of this complex were obtained from acetanilide and phenacetin urines (Smith & Williams, 1949*a*), these traces probably being formed as a result of the production of very small amounts of aniline and phenetidine by deacetylation. It is clear, therefore, that the hypothetical labile glucuronides are only produced from the free aromatic amines and not from the *N*-acetylated amines. Further investigations are in progress on the fate of other types of

acid is derived from labile glucuronides and that the one from *m*-anisidine has some stability.

The structure of the crystalline *p*-toluidine-ammonium glucuronate complex also requires discussion. From its absorption spectrum (see Fig. 1) it is clear that the only absorbing component is *p*-toluidine (according to Dede & Rosenberg (1934) *p*-toluidine shows two absorption bands in water, at 234 μ ., $\log \epsilon$ 3.91 and 288 μ ., $\log \epsilon$ 3.15). Its rising mutarotation in ethanol-water ($[\alpha]_D -60 \rightarrow +2^\circ$) suggests that it is a *p*-toluidide (i.e. a *N*-glucuronide) of β -configuration. On keeping a few weeks the compound turns a light brown. It has no true melting point but decomposes fairly consistently at 125–128°. The analytical data on the compound suggest that it contains 1 molecule of *p*-toluidine, 1 of the *p*-toluidide of ammonium glucuronate and 1 or 1.5 molecules of crystal water. It may be tentatively formulated as

substituted anilines, and so far we have found that *p*-bromoaniline does not give rise in the rabbit to reducing urines. In the case of the naphthylamines, we have found that the 1-derivative gives rise to a urine which reduces Fehling's but not Benedict's reagent, whereas with the 2-derivative the urine only slightly reduced Fehling's solution.

The *p*-toluidine complex cannot be prepared directly from the 'arylamine urine', but is obtained readily from the glucuronide gums prepared at ordinary temperatures by systematic lead acetate precipitations. In the case of *m*-anisidine the complex is only obtained with difficulty from the glucuronide gum and often only after the addition of a little ammonia. The addition of ammonia is unnecessary in the other cases, e.g. aniline and phenetidine. This strongly suggests that the glucuronic

D-Galacturonic acid gives the same type of complex whose properties and spectrum (see Fig. 1) are similar to those of the glucuronic acid complex.

SUMMARY

1. The fate of *p*-phenetidine in the rabbit has been studied, and some observations have been made on the *o*-, *m*- and *p*-anisidines.

2. About 30 % of the phenetidine fed is excreted as ethereal sulphates. The main phenol in the sulphate fraction is 2-hydroxy-4-ethoxyaniline which was characterized as 2-acetoxy-4-ethoxyacetanilide, the synthesis of which is described for the first time. It is suggested that 4-aminoresorcinol is also present.

3. A considerable amount of glucuronide is excreted which appears to be largely a labile glucuronide, the nature of which has not been elucidated.

4. *p*-Acetamidophenylglucuronide has also been isolated from the urine. This substance was obtained in 6% yields, but may occur in the urine to the extent of 20% or more.

5. A *p*-toluidine-ammonium glucuronate complex has been isolated from *p*-phenetidine, *o*-, *m*- and *p*-anisidine urines. The yield from phenetidine urine was high.

6. The *p*-toluidine complexes of ammonium glucuronate and galacturonate have been synthesized and studied, and suggestions have been made regarding their nature.

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A Method for Continuous Graphic Recording of Radioactive Tracer Concentrations from Various Body Regions Simultaneously

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In measuring the changing content of radioactive tracer in different organs of man and animal, it is of great interest to investigate the fate of the tracer at very short time intervals after administration, and also to measure its accumulation simultaneously in several parts of the body. Since information of this kind may not easily be obtained by use of the usual dynatron scalers, certain modifications were necessary to allow continuous recording from different scalers simultaneously.

EXPERIMENTAL

Apparatus

The internal wiring of the dynatron scaler was slightly modified. The connexions to the internal Post Office meter were diverted to a double pole, double throw, changeover switch (*CH*, Fig. 2), thus enabling the operator to switch over to an external 2-pin socket (*S*). Socket and changeover switch were both mounted on the front panel of the scaler. The 2-pin socket facilitated the connexion with an external Post Office meter (*M*), which had the same resistance (2300 Ω) as the meter mounted inside the scaler. It was possible, by these means, to record every tenth or hundredth impulse on either the internal or external Post Office meter. The latter (Fig. 1, and *M*, Fig. 2) was fitted with a small clamping block (*CB*, Fig. 1) on the moving armature, and in this the stem of the writing point (*WP*) was clamped. A brass rod (*R*) was screwed into the back of the meter, permitting the meter to be mounted on a kymograph, where the impulses were continuously recorded.

The Geiger-Müller counters consisted of tungsten helix γ -tubes, made by 20th Century Electronics Co. Ltd. They were shielded with lead tubes in which a $\frac{1}{2}$ in. wide longitudinal slot had been made and the tubes clamped in such a way that the slot was placed just over the organ under investigation. The counters were further shielded with black paper, which nullified the photosensitivity of the tubes. An example of the arrangement of the counters, as used in clinical investigations is illustrated in Fig. 3.

For recording the impulses coming from different Geiger-Müller tubes located on various parts of the body, different scalers were employed, each connected with its external counter writer, thus permitting simultaneous recording of fluctuations of tracer-concentrations.

Clinical investigations

The assembly described above has been so far used clinically for recording changes in ^{131}I concentration only. Prior to the injection of ^{131}I the kymograph was started and the background counts recorded. Then, without stopping the recording mechanism, a dose of ^{131}I was given.

Animal experiments

The method has proved particularly valuable in recording changes of blood ^{131}I concentrations.

A length of antioxidant-free 'Telcothene' tubing (obtained from Telegraph Construction and Maintenance Co. Ltd.) of 1.5 mm. bore was wound round a γ -counter tube in the form of a spiral, which was secured by sticking over with transparent adhesive tape. The total volume of this spiral was approximately 4 ml. (see Fig. 4).

In anaesthetized and heparinized rabbits the carotid was exposed, the blood flow interrupted, and cannulae (*C1* and