Biochemical Studies of Toxic Agents

12. THE BIOSYNTHESIS OF n-PROPYLMERCAPTURIC ACID FROM n-PROPYL HALIDES*

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Before the preliminary account of the present work (Grenby & Young, 1959) there appears to have been no report of the isolation of a biosynthetic alkylmercapturic acid. Evidence had been obtained, however, which suggested that alkylmercapturic acids could probably be isolated from the urine of animals which had been dosed with certain alkylating agents. Roberts & Warwick (1957, 1958), by means of radiocromatography, showed the presence of ethylmercapturic acid in the urine of rats which had been dosed with ethyl methanesulphonate. By chromatographic and radiocromatic methods ethylmercapturic acid was also detected in the urine of rats after administration of bromoethane (Thomson, Maw & Young, 1958), and by the use of a procedure for determining mercapturic acids Bray & James (1958) demonstrated the excretion of alkylmercapturic acids by animals dosed with 1- and 2-bromobutane and certain higher 1-bromoalkanes.

The present work was undertaken to determine whether the three n-propyl halides, 1-chloro-, 1-bromo- and 1-iodo-propane, give rise in the animal body to n-propylmercapturic acid, i.e. N-acetyl-S-(n-propyl)-L-cysteine. 1-Fluoropropane (b.p. -3.2°) was not studied.

\[
\text{NH.CO.CH}_3
\]

\[
\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{X} \rightarrow \text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}
\]

\[
\text{CO.H}
\]

(X = Cl, Br or I)

By means of paper chromatography n-propylmercapturic acid was detected in the urine of rats, rabbits, guinea pigs and mice dosed with 1-bromo- or 1-iodo-propane, and in the urine of rats and rabbits dosed with 1-chloropropane. The mercapturic acid was isolated from the urine of rats which had been dosed with 1-bromo- or 1-iodo-propane.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected.

* Part 11: Knight & Young (1958).

Preparation of S-(n-propyl)-L-cysteine

Method I. The procedure was based on that described by Armstrong & Lewis (1951) and depended on the reduction of L-cystine by sodium in anhydrous ammonia (du Vigneaud, Audrieth & Loring, 1930) followed by S-propylation of the cysteine by means of 1-iodopropane. Small pieces of metallic sodium were added, with stirring, to 2 g. of L-cystine dissolved in 100 ml. of anhydrous ammonia cooled in a mixture of solid CO\(_2\) and ethanol, until a permanent deep-blue colour was produced. With continued stirring 0-2-0.4 ml. portions of 1-iodopropane were added at intervals of about 5 min. until a sample of the reaction mixture gave a negative nitroprusside test for the presence of thiogroups. 2-2 ml. of 1-iodopropane being required to achieve this. The ammonia was allowed to evaporate at room temperature, leaving a white residue which was then dissolved in the minimum volume of water. The pH of the solution was adjusted to 5-0 by the addition of conc. HCl and the precipitate which formed was separated and crystallized first from 85% (v/v) methanol in water and then from water. The product consisted of colourless glistening plates of S-(n-propyl)-L-cysteine in an amount corresponding to 65% of the theoretical yield; m.p. 241-243° (decomp.); \([\alpha]_D^{22} -22°\) in water (c, 2); \([\alpha]_D^{245} -5°\) in n-HCl (c, 1) (Found: C, 44.4; H, 8.1; N, 8.6; S, 19.3. Calc. for C\(_4\)H\(_9\)O\(_2\)NS: C, 44.1; H, 8.0; N, 8.6; S, 19.6%). The compound was soluble in water to the extent of 2-5 g./100 ml. at 22°, but was only very sparingly soluble in the common organic solvents.

Method II. This method was similar to that of Stoll & Seebeck (1948) and Roberts & Warwick (1958), and involved the interaction of 1-iodopropane and L-cysteine in an aqueous ethanol solution of NaOH. The yield of S-(n-propyl)-L-cysteine was 21%; m.p. 245-247° (decomp.); \([\alpha]_D^{22} -22°\) in water (c, 2) (Found: C, 44.3; H, 7.9; N, 8.6; S, 19.8%).

Preparation of n-propylmercapturic acid

Method I. To 3 g. of S-(n-propyl)-L-cysteine in 9 ml. of water was added 4.5 ml. of 5 N-NaOH, and the solution was cooled to just below 5°. The temperature was maintained at this level while a steady stream of keten was bubbled through the solution. When necessary, drops of 5N-NaOH were added to keep the solution just alkaline to phenolphthalein. After keten had passed through the solution for 30 min. the solution no longer gave a positive ninhydrin test. It was then made to pH 2-0 by the addition of conc. HCl and extracted by shaking three times with three-volume portions of chloroform. The chloroform extracts were combined and, when evaporated to dryness, yielded
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a gummy residue which gradually solidified. This was crystallized twice from a small volume of water and twice from benzene; the yield was 65% of the theoretical amount of n-propylmercapturic acid. The product was in the form of needles, m.p. 96°; [α]20D = 19° in water (c, 2) (Found: C, 46-6; H, 7-2; N, 6-7; C8H15O4NS requires C, 48-8; H, 7-4; N, 6-8; S, 15-6%).

Method II. The procedure resembled that described by du Vigneaud, Wood & Binkley (1941) for the acetylation of p-bromophenyl-L-cysteine by acetic anhydride in the presence of NaOH. Starting from 0-267 g. of S-(n-propyl)-L-cysteine the yield of mercapturic acid was 57%; m.p. 95°; [α]20D = 18° in water (c, 2) (Found: C, 46-7; H, 7-3; N, 7-1; S, 15-9%).

Method III. N-Acetyl-L-cysteine, m.p. 110°, was prepared by a method based on that of Pirie & Hele (1933), in which L-cystine is acetylated by ketene and is then reduced to N-acetyl-L-cysteine. n-Propylmercapturic acid was prepared by allowing 3 ml. of 1-iiodopropane to react with 2-2 g. of N-acetyl-L-cysteine in anhydrous ammonia under conditions similar to those already described under method I for the preparation of S-(n-propyl)-L-cysteine. The yield was 72%; m.p. 96°; [α]20D = 19° in water (c, 2) (Found: C, 47-2; H, 7-1; N, 6-9; S, 15-4%).

n-Propylmercapturic acid had the following solubilities at 20° (in g./100 ml. of solution): water, 5-6; ethanol, 28-3; ether, 2-7; chloroform, 24-0; benzene, < 0-1.

Deacetylation of n-propylmercapturic acid

A step which was used in the identification of n-propylmercapturic acid in the present work was its conversion into S-(n-propyl)-L-cysteine. This deacetylation was brought about by acid hydrolysis and also enzymically.

By acid hydrolysis. To 0-151 g. of synthetic n-propylmercapturic acid were added 2 ml. of water and 8 ml. of conc. HCl. The solution was heated in a boiling-water bath for 5 hr. and was then concentrated until crystallization began. The hydrolysate was made to pH 5-0 at room temperature by the addition of NaOH solution, concentrated again and left in the refrigerator. The plates which separated were recrystallized from a small volume of water, filtered and washed with water and with acetone. The yield of S-(n-propyl)-L-cysteine was 33% of the theoretical; m.p. 239-241° (decomp.); the mixed m.p. with the authentic compound was 239-240° (decomp.); [α]20D = 22° in water (c, 2) (Found: C, 44-5; H, 8-1; N, 8-7; S, 19-8%).

By enzymic hydrolysis. A variety of acetamido compounds have been shown to undergo deacetylation when incubated with tissue extracts (see Bray, James, Thorpe & Wasdell, 1950). The enzymic deacetylation of N-acetyl-S-benzyl-L-cysteine was demonstrated by Birnbaum & Greenstein (1952). In the present work it was found that when n-propylmercapturic acid was incubated with an extract of the liver or kidney of the rat it was deacetylated. The preparation of the tissue extract and the conditions of hydrolysis were similar to those described by Bray, James, Rawlin & Thorpe (1949) for the enzymic hydrolysis of amidines.

The liver (7-3 g.) of a freshly killed rat was ground with a little fine sand and about twice its weight of water, and centrifuged at 1000 g. for 15 min. The supernatant liquid was decanted and added to a solution of 0-496 g. of n-propylmercapturic acid in 50 ml. of water, 8 ml. of 0-2N-Na2HPO4 and 2 ml. of 0-2N-NaH2PO4. The mixture was adjusted to pH 7-4 and incubated at 37° for 24 hr. It was boiled and filtered, and the filtrate was shaken first with an equal volume of chloroform, then with an equal volume of ether. The aqueous solution was evaporated until crystals appeared and was left in the refrigerator. The precipitate was recrystallized twice from water, filtered and washed with acetone. The product consisted of plates in a yield corresponding to 12% of the theoretical, m.p. 242-243° (decomp.) (Found: C, 44-3; H, 7-5; N, 8-5; S, 20-0%). No such product was obtained in control experiments in which either the tissue extract or n-propylmercapturic acid was omitted. The conditions for deacetylation of the mercapturic acid by extracts of rat kidney were similar to those described for liver.

Cleavage of n-propylmercapturic acid by alkali

Although fairly resistant to acid hydrolysis, n-propylmercapturic acid is readily broken down by NaOH, with formation of the sodium salt of n-propanethiol. This thiol can be characterized as n-propanethiol mercury chloride, CH3-CH2-CH2-S-HgCl (Challenger & Rawlings, 1937), which was prepared by mixing 0-42 ml. of n-propanethiol with 12 ml. of 15% (w/v) mercuric chloride in methanol. The mixture was kept for 24 hr. with occasional shaking and the white precipitate which formed was filtered and crystallized from a large volume of ethanol. Small sparking plates were obtained in a yield corresponding to 43% of the theoretical, m.p. 181° (decomp.). Challenger & Rawlings (1937) reported that the compound melted at 182° (decomp.) (Found: C, 11-4; H, 2-4; S, 10-4. Calc. for C2H13S0Hg: C, 11-6; H, 2-3; S, 10-5%).

A solution of 0-408 g. of n-propylmercapturic acid in 5 ml. of 15% (w/v) NaOH in water was heated in a water bath at 90° for 15 min. The yellow solution thus obtained was cooled in ice, and conc. HCl was added drop by drop until the solution became turbid and the odor of the thiol markedly increased. On the addition of 6 ml. of 15% (w/v) mercuric chloride in methanol a pale-yellow solid appeared. This was crystallized from ethanol and gave white crystals in a yield corresponding to 24% of the theoretical; m.p. 182° (decomp.), unchanged by admixture with authentic n-propanethiol mercury chloride prepared as already described (Found: C, 11-8; H, 2-2; S, 10-3%).

Animals and dosing

Rats, rabbits, guinea pigs and mice were housed in metabolism cages so constructed that the urine was collected separately from the faeces. Urine was collected for 24 hr. after dosing, or for 48 hr. from rabbits, and when not used immediately it was stored in the refrigerator. Rats, rabbits and mice were given a diet of rat cakes [J. Murray and Sons (London) Ltd.] and had access to water at all times. The rabbits received cabbage in addition. Guinea pigs were fed on a mixture of bran and oats and were also given cabbage.

1-Chloropropane, 1-bromopropane and 1-iodopropane (British Drug Houses Ltd.) were redistilled. They were administered to the animals, either undiluted or as a 40% (w/v) solution in arachis oil or liquid paraffin, by subcutaneous injection in the dorsal region. With the exception of the rabbits, the animals were anaesthetized lightly with ether whilst being dosed.
Chromatographic studies

In early experiments the urine of each group of animals dosed with an n-propyl halide was examined by paper chromatography for the presence of n-propylmercapturic acid. In later work concentrates of chloroform extracts of the acidified urine were used instead so as to reduce interference by other urinary constituents.

General methods. Urine extracts were prepared by acidifying the urine with HCl to pH 2-0 and shaking it three times with three-volume portions of chloroform. The combined chloroform extracts were evaporated to a small volume, and suitable amounts of the concentrate were applied to the paper for testing for the presence of the mercapturic acid. Some of the chloroform extract was evaporated to dryness and subjected to acid hydrolysis under the conditions described earlier, with appropriate reduction in volumes. The hydrolysate was examined by paper chromatography as described in the text. Detection of S-(n-propyl)-L-cysteine might appear in the hydrolysate as a result of the breakdown of compounds other than the mercapturic acid.

It was recognized, however, that whereas the reagent was less satisfactory than the K$_2$Cr$_2$O$_7$-AgNO$_3$ reagent.

Detecting reagents. Two reagents were used, both of which detect n-propylmercapturic acid and S-(n-propyl)-L-cysteine. One of these was the K$_2$Cr$_2$O$_7$-AgNO$_3$ reagent described by Knight & Young (1958) and the other was 0-1 n-KMnO$_4$. When the chromatogram was sprayed lightly with 0-1 n-KMnO$_4$ a pink background was produced with yellow areas marking the positions of the mercapturic acid and the S-(n-propyl)-L-cysteine. The colours faded within a few minutes, however, and in this respect this reagent was less satisfactory than the K$_2$Cr$_2$O$_7$-AgNO$_3$ reagent.

Solvent mixtures. (A) The upper layer obtained after shaking a mixture of equal parts of butan-1-ol (AnalaR) and 1:33 n-acetic acid. (B) Pyridine-water-aq. NH$_3$ soln.

Results. Male rats, rabbits, guinea pigs and mice were injected subcutaneously with a 40 % (w/v) solution of the 1-halogenopropane in arachis oil and their urine was examined by means of paper chromatography for the presence of n-propylmercapturic acid (see Table 1).

Samples of the normal urine of each species studied, or of the urine excreted after the administration of the diluent alone, showed no evidence for the presence of n-propylmercapturic acid.

Isolation of n-propylmercapturic acid from the urine of rats dosed with 1-bromopropane

After administration of 1-bromopropane dissolved in a diluent. A 40 % (w/v) solution of 1-bromopropane in liquid paraffin was injected subcutaneously into 18 rats (150-200 g. body wt.) so that each received 0-25 g. of the halogenopropane. The dosing was repeated on three further occasions at intervals of 48 hr. and the urine excreted in the 24 hr. immediately following each dosing was collected. The urine, total volume 520 ml., was filtered and made acid to Congo red by the addition of conc. HCl. The acidified urine was shaken on a mechanical shaker for 30 min. with 3 vol. of chloroform and the extract was separated after centrifuging to break the emulsion. The extraction was repeated twice more and the combined chloroform extracts were then evaporated to 50 ml. The dark-red solution thus obtained was shaken with 20 ml. of NaHCO$_3$, then with 10 ml. of NaHCO$_3$. The combined bicarbonate extracts were brought to pH 2-0 by the addition of conc. HCl, and the acidified solution was shaken three times with 3 vol. of chloroform. These chloroform extracts were evaporated to dryness and, after standing over P$_2$O$_5$, yielded 1-197 g. of dark-brown gum. When this was stirred with a few millilitres of ether a buff-coloured solid formed. This was separated by filtration and washed with a small volume of ether. When the solid (0-438 g.) was treated with 5 ml. of ethyl acetate part went into solution, leaving undissolved material which was separated by filtration and identified as hippuric acid. Light petroleum (b.p. 40-60°) was added.

Table 1. Excretion of n-propylmercapturic acid by animals dosed with 1-halogenopropanes

All the animals were dosed with a 40 % (w/v) solution of the 1-halogenopropane in arachis oil by subcutaneous injection in the dorsal region. The urine was examined by paper chromatography as described in the text. Detection of mercapturic acid: + +, readily detected; +, detected with difficulty; -, not detected.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body wt. (g.)</th>
<th>1-Halogenopropane administered</th>
<th>Dose (g./kg. body wt.)</th>
<th>n-Propylmercapturic acid in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>150-200</td>
<td>Chloro-</td>
<td>1-5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bromo-</td>
<td>1-5</td>
<td>+</td>
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<td></td>
<td></td>
<td>Iodo-</td>
<td>1-0</td>
<td>+</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2400-3200</td>
<td>Chloro-</td>
<td>1-3</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Bromo-</td>
<td>1-0</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Iodo-</td>
<td>1-2</td>
<td>+</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>300-450</td>
<td>Chloro-</td>
<td>1-8</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>Bromo-</td>
<td>1-8</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Iodo-</td>
<td>1-3</td>
<td>+</td>
</tr>
<tr>
<td>Mouse</td>
<td>24-28</td>
<td>Chloro-</td>
<td>1-2</td>
<td>-</td>
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<td></td>
<td></td>
<td>Bromo-</td>
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<td></td>
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<td>Iodo-</td>
<td>1-2</td>
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</tbody>
</table>
drop by drop to the ethyl acetate solution with stirring, and dark gummy material separated and adhered to the sides of the beaker. When enough light petroleum had been added to render the solution almost colourless, the clear solution was decanted and more light petroleum was added with stirring. This yielded 0.216 g. of an almost white solid, which was crystallized from benzene. The crystals were dissolved in ethanol and the solution was treated with charcoal and filtered. The filtrate was evaporated to dryness and the residue was crystallized from water. This yielded 0.081 g. of needles, m.p. 95°, unchanged by admixture with synthetic n-propylmercapturic acid; [α]D20 = 19° in water (c, 2) (Found: C, 46.9; H, 7.0; N, 7.1; S, 15.6%). The Rf values of the compound in solvent mixtures (A) and (B) were the same as those of synthetic n-propylmercapturic acid run on the same chromatograms. When the compound was hydrolysed with HCl the hydrolysate gave chromatograms corresponding to those of S-(n-propyl)-L-cysteine.

In another experiment 33 rats (250–280 g. body wt.) were each injected subcutaneously on two occasions with 0.25 g. of 1-iodopropane. From the urine excreted in the 24 hr. period after dosing, 0.229 g. of crystalline product was isolated by the method already described, m.p. 95°, unchanged by admixture with synthetic n-propylmercapturic acid; [α]D20 = 19° in water (c, 1) (Found: C, 46.6; H, 7.4; N, 6.8; S, 15.9%). A portion (0.190 g.) of the isolated compound was decrystallized by incubation with a tissue extract as described earlier, except that rat kidney (1-7 g.) instead of liver was used. A crystalline product was separated weighing 0.050 g., m.p. 236–238° (decomp.); m.p. when mixed with synthetic S-(n-propyl)-L-cysteine 237–239° (decomp.); [α]D20 = 21° in water, (c, 1) (Found: C, 44.2; H, 7.9; N, 8.7; S, 19.7%).

When the procedure for isolating the mercapturic acid was applied to 555 ml. of normal rat urine, this compound was not found.

**Attempted isolation of n-propylmercapturic acid from the urine of rats dosed with 1-chloropropane**

As the results obtained by chromatographic examination of the urine of rats dosed with 1-chloropropane indicated the presence of a low concentration of n-propylmercapturic acid, an attempt was made to isolate the mercapturic acid from the urine. Each rat of a group of 60 (190–240 g. body wt.) was injected subcutaneously on three occasions with 0.25 g. of 1-chloropropane, each rat being dosed with 1-chloropropane and acidified and extracted with chloroform. The chloroform extracts gave evidence of the presence of n-propylmercapturic acid when examined by paper chromatography. When they were fractionated as already described, however, no product was obtained at the point in the process at which n-propylmercapturic acid was isolated from the urine of rats dosed with 1-bromo- or 1-iodo-propane.

**DISCUSSION**

The physical and chemical properties of the compound isolated from the urine of rats which had been dosed with 1-bromo- or 1-iodo-propane compared with those of the authentic mercapturic acid, clearly establish that the isolated compound was n-propylmercapturic acid. The compound was isolated from urine which had been acidified, however, and it is known that some mercapturic acids are not excreted as such, but are derived from...
precursors which are broken down when the urine is acidified. These acid-labile precursors of mercapturic acids have been designated ‘premercapturic acids’ (Knight & Young, 1958) and that formed from naphthalene has been isolated and identified as \( N\)-acetyl-S-(1:2-dihydro-2-hydroxynaphthyl)-L-cysteine by Boyland & Sims (1958). All the compounds shown so far to give rise to premercapturic acids in vivo are aromatic, however, and for the aralkyl compound, benzyl chloride, there is evidence that it is excreted as a mercapturic acid and not as a premercapturic acid (Knight & Young, 1958). At present there are no grounds for believing that an alkyl halide is converted into an acid-labile compound from which the alkylmercapturic acid is liberated. This is supported by the observation that when the unacidified urine of animals which had been dosed with a 1-halogenopropane was examined by paper chromatography with a basic solvent system, pyridine–water–ammonia solution (sp.gr. 0·88) (20:2:1), a spot was obtained with \( R_c \) corresponding to that of \( n \)-propylmercapturic acid run on the same chromatogram. In the absence of evidence to the contrary, it would seem reasonable therefore to assume that \( n \)-propylmercaptopuric acid is a constituent of the urine of rats after the administration of 1-bromo- or 1-iodo-propane.

An interesting feature of work on the hydrolysis of \( n \)-propylmercapturic acid was the observation that this compound undergoes deacetylation when incubated with extracts of the liver or kidney of the rat. Evidence that deacetylation of a mercapturic acid can occur in vivo was obtained by Parke & Williams (1951), who reported the excretion of \( S \)-phenyl-L-cysteine by rabbits to which phenylmercapturic acid had been administered. The finding that enzymic deacetylation of \( n \)-propylmercapturic acid can occur raises the question of whether this has a bearing on the amount of the mercapturic acid excreted by animals dosed with a 1-halogenopropane. There is a possibility that this deacetylation process occurs only to a small extent in the intact animal, and that it is favoured by the conditions under which it is studied in vitro; evidence that such a situation can occur was obtained by Krebs, Sykes & Bartley (1947) in their study of the deacetylation of the \( p \)-acetamido group of sulphonamide drugs by animal tissues.

The amounts of \( n \)-propylmercapturic acid isolated from the urine represented the conversion of from 0·27 to 1·14% of the administered halogenopropane to the mercapturic acid. Even allowing for the fact that the isolation process was carried out with the object of obtaining an analytically pure product, it would seem that mercapturic acid formation accounts for the fate of no more than a small proportion of the administered halogenopropane. This may be attributable to other factors in addition to the influence of competing metabolic reactions. For example, it is probable that the volatility of the 1-halogenopropanes leads to some loss of the compounds through the lungs. This is of special significance with respect to 1-chloropropane, b.p. 46·6°, and could help to explain the low concentration of \( n \)-propylmercapturic acid in the urine of rats and rabbits dosed with 1-chloropropane, and the apparent absence of the mercapturic acid from the urine of guinea pigs and mice dosed with this compound. In view of this finding with guinea pigs it is of interest that Bray, Franklin & James (1959) have pointed out that mercapturic acid excretion by the guinea pig is much lower than that by the rat and rabbit under similar conditions.

Current interest in the biochemistry of alkylating agents stems mainly from the variety of biological effects which they produce and their significance in relation to the cancer problem. Their metabolism is being studied in the belief that the information gained may help to explain their action in the organism. The biosynthesis of \( n \)-propylmercapturic acid from a 1-halogenopropane provides a clear-cut example of the alkylation of the thiol group in vivo.

**SUMMARY**

1. \( S \)-(\( n \)-Propyl)-L-cysteine has been prepared by two methods, and \( n \)-propylmercapturic acid, i.e. \( N \)-acetyl-\( S \)-(\( n \)-propyl)-L-cysteine, by three methods.
2. The hydrolysis of \( n \)-propylmercapturic acid by acid and by alkali has been studied, and its conversion into \( S \)-(\( n \)-propyl)-L-cysteine by incubation with extracts of rat liver or kidney has been demonstrated.
3. By means of paper chromatography evidence has been obtained for the excretion of \( n \)-propylmercapturic acid by rats, rabbits, guinea pigs and mice dosed with subcutaneous injection with 1-bromopropane or 1-iodopropane, and by rats and rabbits, but not by guinea pigs and mice, injected subcutaneously with 1-chloropropane.
4. \( n \)-Propylmercapturic acid has been isolated from the urine excreted by rats in the 24 hr. after they had been injected subcutaneously with 1-bromopropane or 1-iodopropane.

The authors are grateful for the support the work has received from the Endowment Fund of St Thomas’s Hospital. They wish to thank Mr A. R. Morrison for performing a number of the sulphur analyses reported in the paper; other elementary microanalyses were carried out by Weiler and Strauss, Oxford. A preliminary account of the work was presented at a meeting of the Biochemical Society held at St Thomas’s Hospital Medical School on 23 January 1959 (Grenby & Young, 1959).
The Effect of Growth on the Composition of Avian Muscle

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Muscular growth in birds and mammals is known to involve a fall in the percentage of water and extracellular electrolytes, and a rise in the percentage of protein, potassium and phosphorus (Leslie & Davidson, 1951; Robinson, 1952a; Barlow & Manery, 1954). McCance & Widdowson (1956) found that during the development of human and pig muscle there was an increase in the nitrogen/potassium ratio. This was interpreted to mean that development might be associated with a change in the gross composition of the muscle cell. The work on these two mammals has been extended and the results are reported elsewhere (Dickerson & Widdowson, 1960). The present paper describes observations of a similar nature on avian muscle.

EXPERIMENTAL

Birds used in this investigation were pure-bred Rhode-Island Red cockerels. Twenty-four birds were taken as soon as possible after they had hatched. A further 12 birds aged 2-3 weeks (wt. approx. 100 g.), nine aged 4 weeks (wt. approx. 200 g.) and four aged 27 weeks (wt. approx. 3.5 kg.) were used. The 27-week-old birds were assumed to be adult for the present purpose.

Methods

Newly hatched chicks. These were divided into three groups, each of eight animals, in such a way that the average weight of the birds in each group was approximately the same. The pectoral muscles of all the birds in each group were pooled for analysis. The birds aged 2-3 weeks and 4 weeks were also each divided into three groups and treated in a similar way. The muscle from each adult bird was analysed separately.

Young birds. These were killed by a blow on the head and about 1 ml. of blood was taken by cardiac puncture from each, and this clotted satisfactorily under liquid paraffin. The samples taken from the individual birds in each group were pooled so that three pooled samples of serum were obtained at each of these ages. The pectoral and sartorius muscles of each bird were dissected as completely as possible and placed in tared specimen tubes which had been previously cooled on solid CO₂. The frozen muscle was weighed.

Adult birds. These were killed by the injection of 180 mg. of pentobarbitone sodium (Nembutal). About 5 ml. of blood was taken by heart puncture before the animal died and allowed to clot under paraffin. Further haemorrhage was avoided (Widdowson & Southgate, 1959). The pectoral muscles of one side and the sartorius muscle of both sides were dissected as completely as possible, weighed at once and representative samples frozen on solid CO₂. The weight of the pectoral muscles of these animals was obtained by multiplying the weight taken from one side by two. A small piece of muscle from one animal of each age and kind was fixed in formalin-0.9% NaCl for histological examination. In preparation for analysis, the pectoral muscle was thawed, freed of visible fat and tendon, and thoroughly cut up with scissors.

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