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## DETERMINATION OF THIOCYANATE IN TISSUES AND BODY FLUIDS OF ANIMALS BY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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### SUMMARY

A method for the quantitative extraction of thiocyanate from biological material has been developed. Levels of 0.2–5 ppm of thiocyanate, in the presence of cyanide, were determined by gas-solid chromatography using 2-bromopropane as internal standard.

Reliable results can be obtained only after careful deproteinization of the extracts with hot methanol. Cyanide can be eliminated by reaction with alkaline formaldehyde. The reproducibility of the determination of thiocyanate in biological extracts was  $\pm 5\%$ ; the recoveries were over 90%.

*In vivo* experiments with  $\text{KS}^{14}\text{CN}$ -treated rats gave good agreement between the analytical results and the amount of thiocyanate determined by isotope dilution.

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### INTRODUCTION

The thyreostatic effect of the thiocyanate ion, produced by inhibiting iodine transport in the thyroid, is well known<sup>1</sup>. Contrary to most thyreostatic drugs, the thiocyanate ion is present as a normal constituent in mammalian tissues and body fluids<sup>2</sup>. The thiocyanate levels may vary widely<sup>3</sup>, depending on the dietary ingestion of the ion or its esters and other precursor compounds such as cyanide, nitriles and isothiocyanates<sup>4</sup>. The glucosinolates of the *Cruciferae*, such as glucobrassicin and neoglucobrassicin, are the most important sources of thiocyanate in the food of ruminants.

The use of thiocyanate as a thyreostatic drug can be controlled if a suitable method for its determination in biological fluids is available and if the normal tissue levels are known.

Many of the methods described for the determination of thiocyanate cannot be applied to biological samples. Measurement of the normal thiocyanate levels in plasma, saliva and urine by formation of a red-brown complex with  $\text{Fe}^{3+}$  (refs. 5–7) or a green complex with  $\text{Cu}^{2+}$  and pyridine<sup>8</sup> approaches the detection limit of these methods.

Better results can be obtained by the conversion of thiocyanate via cyanide into a cyanogen halide and reaction of the latter with pyridine or nicotinamide to give a glutacondialdehyde. The colour of the aldehyde is measured either directly<sup>9</sup> or after reaction with benzidine<sup>10</sup>, pyrazolone<sup>11</sup> or *p*-phenylenediamine<sup>12</sup>. The sample can be distilled and the cyanide trapped in potassium hydroxide<sup>13</sup> or alternatively by diffusion in a Conway vessel<sup>14</sup>. The methods mentioned above are not suitable for daily routine analyses because they are very time consuming.

Some workers have reported the determination of thiocyanate by solvent extraction with Rhodamine B<sup>15</sup> or methylene blue<sup>16</sup>. The application of these methods to biological samples failed owing to interferences<sup>17</sup>. Interfering substances can probably be separated from thiocyanate by paper chromatography<sup>18</sup> but quantitation is difficult<sup>17</sup>.

Nota and Palombari<sup>19</sup> reported a gas-solid chromatographic (GSC) determination of thiocyanate and cyanide in waste water. After investigation of this method, a modification was developed for application to biological samples.

## EXPERIMENTAL

### *Reagents and reference compounds*

Bromine, orthophosphoric acid, potassium thiocyanate and sodium cyanide were obtained from Merck (Darmstadt, G.F.R.). Benzene and methanol were purchased from J. T. Baker (Deventer, The Netherlands).

Porapak Q (80–100 mesh) was obtained from Waters Assoc. (Milford, Mass., U.S.A.) and potassium [<sup>14</sup>C]thiocyanate from the Radiochemical Centre (Amersham, Great Britain).

Methyl Cellosolve (ethylene glycol monoethyl ether) of Sequanal grade was purchased from Pierce (Rockford, Ill., U.S.A.); PPO (2,5-diphenyloxazole), dimethyl-POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene] and Soluene TM 100 were obtained from Packard (La Grange, Ill., U.S.A.).

All other reagents were reagent-grade products from different manufacturers.

### *Solutions*

Alkaline formaldehyde solution was prepared by mixing 4 ml of 40% formaldehyde solution with 16 ml of distilled water and 0.4 ml of 1 *N* sodium hydroxide solution. Bromine-water was prepared by saturating distilled water with bromine. Benzene internal standard solution was prepared by adding 0.01 ml of 2-bromopropane to 1 l of benzene and the concentration of the solution was adjusted to the desired value (2–3 ppm SCN<sup>-</sup>) by dilution with benzene. Fe<sup>3+</sup>/Fe<sup>2+</sup> reagent was prepared by dissolving 1 g of FeCl<sub>3</sub> and 1 g of FeSO<sub>4</sub> in and adjusted the volume to 100 ml with 0.1 *N* hydrochloric acid.

### *Standard solutions*

A 1000 ppm stock solution of SCN<sup>-</sup> or CN<sup>-</sup> was prepared by dissolving 167.3 mg of KSCN or 188.4 mg of NaCN, respectively, in 100 ml of doubly distilled water. Standard solutions of concentration 1–10 ppm were prepared by dilution of the stock solutions with doubly distilled water.

*Chromatographic conditions*

The gas chromatograph used was a Hewlett-Packard Model 5750 equipped with a pulsed  $^{63}\text{Ni}$  electron-capture detector (ECD). The pulse interval of the ECD was 15  $\mu\text{sec}$ . The glass chromatographic column (2 m  $\times$  2 mm I.D.) was packed with Porapak Q (80–120 mesh). The flow-rate of the argon–methane (90:10) carrier gas was 50 ml/min. The column, injection port and detector temperatures were 155°, 180° and 230°, respectively.

*Analytical procedure*

A tissue sample (0.5 g) is homogenized with 5 ml of methanol with an Ultra-turrax and centrifuged at 10,000 rpm for 10 min (12,000 g). Fluid samples are de-proteinized by homogenization of 1 ml of liquid with 4 ml of methanol. The methanol extract is boiled under reflux for 5 min, then cooled and centrifuged. An aliquot of the supernatant is adjusted to 0.5 ml with distilled water and mixed with 0.5 ml of alkaline formaldehyde solution. After 5 min the cyanide reaction with formaldehyde is complete and 0.7 ml of orthophosphoric acid solution followed by 0.3 ml of bromine-water are added. After a reaction time of 5 min, a spatula-tip amount (ca. 50 mg) of ascorbic acid is added and the reaction mixture is shaken until the brown colour of bromine disappears. The mixture is then extracted with 2 ml of benzene internal standard solution and a 1–3- $\mu\text{l}$  aliquot of the benzene layer is injected into the gas chromatograph.

*Spectrophotometric determination of thiocyanate in urine*

The method described is a modification of an  $\text{Fe}^{2+}/\text{Fe}^{3+}$  method<sup>6</sup>. A 3-ml volume of urine is mixed with 1 ml of 6 N hydrochloric acid and extracted with 4 ml of a mixture of diethyl ether and 2-butanone (1:1, v/v). The upper layer is removed and re-extracted with 4 ml of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  reagent. The extinction is measured at 455 nm.

*Determination of  $^{14}\text{C}$ -labelled thiocyanate*

A Packard Tri-Carb 3003 liquid scintillation counter was used with toluene–methyl Cellosolve (4:1, v/v) containing 1.2% of PPO and 0.004% of dimethyl-POPOP as the scintillation liquid. Tissue samples were dissolved in Soluene at 60° (0.1:1, w/v). From the liquid samples an aliquot corresponding to 10% of the counts was taken. After evaporation of the volatile solvents by a stream of nitrogen, 20 ml of the scintillation liquid were added and the vials placed in the liquid scintillation counter. The values were corrected for background activity and quenching.

## RESULTS AND DISCUSSION

*Optimization of the analytical procedure*

Nota and Palombari<sup>19</sup> described the reaction of cyanide and thiocyanate to give cyanogen bromide. Mixing the solution with a 50-fold excess of 40% formaldehyde solution was found to inhibit the reaction of cyanide. Excess of bromine was removed with 5% aqueous phenol solution. This mixture was injected directly into the gas chromatograph.

This procedure was examined but interfering peaks were obtained on the

chromatogram owing to the presence of phenol. However, removing the excess of bromine with solid ascorbic acid eliminated this interference without changing the sample volume. The binding of cyanide to a 50-fold excess of formaldehyde was found to be unsatisfactory: the sample is diluted 50-fold and the large amount of formaldehyde causes interfering peaks. As the formation of a cyanohydrin from cyanide and an aldehyde is base catalysed<sup>20</sup>, addition of an equal volume of 8% alkaline formaldehyde to the sample resulted in complete removal of the cyanide ion within 5 min.

Direct injection of aqueous cyanogen bromide solutions, derived from biological samples, into the column resulted in the appearance of peaks with high retention times, which interfered with the next chromatogram. Extraction of the cyanogen bromide solution with some organic solvents overcame this difficulty. The extraction yields and the distribution coefficients of cyanogen bromide between aqueous and organic layers were studied using  $\text{KS}^{14}\text{CN}$ . To 0.5 ml of a standard solution containing 2 ppm of thiocyanate  $5 \cdot 10^4$  cpm of  $\text{KS}^{14}\text{CN}$  was added, the procedure was carried out as described and a 200- $\mu\text{l}$  aliquot of both phases was counted. From the distribution coefficients obtained (Table I), it can be calculated that *ca.* 30% of the cyanogen bromide was extracted with aliphatic hydrocarbons. Extraction of cyanogen bromide with benzene or toluene resulted in a 80% extraction. However, the highest extraction yields were obtained with ethers, alcohols and ketones. Nevertheless, benzene (or toluene) was chosen as the extraction solvent as a satisfactory extraction yield and a low response of the solvent on the ECD was obtained.

TABLE I

DISTRIBUTION COEFFICIENTS (*D*) OF CYANOGEN BROMIDE BETWEEN AQUEOUS AND ORGANIC LAYERS

| <i>Solvent</i>                   | <i>D</i> | <i>Solvent</i>             | <i>D</i> |
|----------------------------------|----------|----------------------------|----------|
| <i>n</i> -Hexane                 | 0.6      | Diethyl ether              | 9.3      |
| <i>n</i> -Heptane                | 0.4      | Diisopropyl ether          | 4.6      |
| Isooctane                        | 0.4      | <i>n</i> -Butanol          | 7.7      |
| Light petroleum<br>(b.p. 40–60°) | 0.7      | <i>n</i> -Pentanol         | 6.8      |
| Benzene                          | 4.9      | <i>tert.</i> -Amyl alcohol | 11.5     |
| Toluene                          | 5.6      | 2-Butanone                 | 6.7      |

Quantitative determination of thiocyanate was performed using an internal standard added to the extraction solvent. Aliphatic bromides were studied because of their analogy with cyanogen bromide during extraction and response on the ECD. The relative retention times of some aliphatic bromides under the GSC conditions used were cyanogen bromide, 1.00; methyl bromide, 0.53; ethyl bromide, 1.17; 2-bromopropane, 2.22; and 1-bromopropane, 2.50. In examining biological samples, the best results were obtained with 2-bromopropane as internal standard. Chromatograms of a 2 ppm thiocyanate standard solution and of a meat extract with 2-bromopropane as internal standard are shown in Fig. 1.

#### Calibration graph

The analytical procedure was carried out on 0.5-ml standard solutions con-

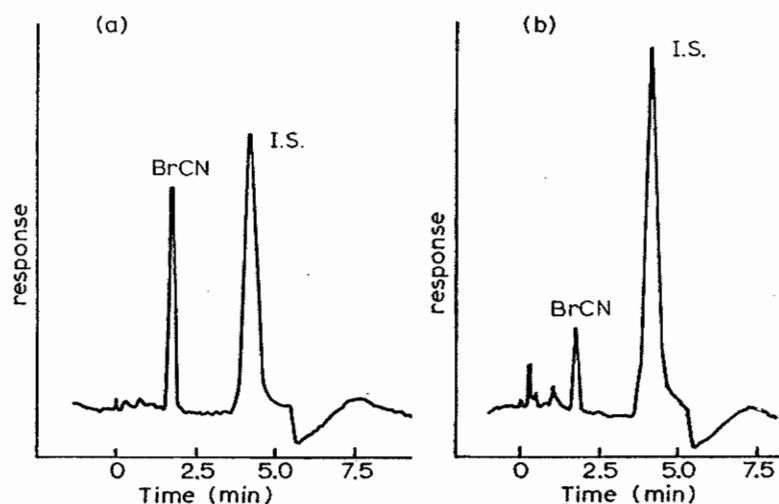


Fig. 1. (a) Gas chromatogram of BrCN obtained from an aqueous standard thiocyanate solution of 2 ppm. (b) Gas chromatogram of BrCN obtained from a meat extract (*Longissimus dorsi*). I.S. = internal standard.

taining 1–5 ppm of thiocyanate. The peak height of cyanogen bromide divided by the peak height of the internal standard was plotted against the amount of thiocyanate, and the linear calibration graph obtained is shown in Fig. 2. The correlation coefficients were better than 0.994 in all instances. The standard deviation of the individual values was 4.9% at the 5 ppm level and 13.2% at the 1 ppm level. The limit of detection, under the conditions used, was 0.2 ppm.

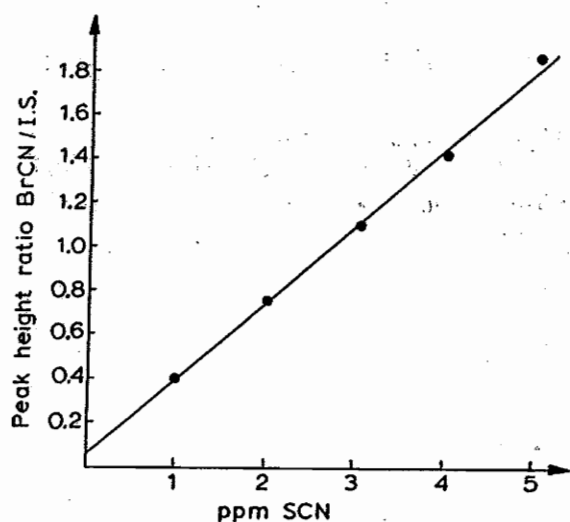


Fig. 2. Calibration graph for thiocyanate.

#### *Response of proteins in thiocyanate determination*

Direct bromine treatment of skim milk or plasma, compared with samples deproteinized with methanol, resulted in 60-fold higher thiocyanate levels (Table II). The anomalous protein response could be due to cyanogen bromide or to some unknown substance generated by reaction of bromine with a protein. This aspect was studied using both GSC and a spectrophotometric method<sup>12</sup>. As similar results were

TABLE II

THIOCYANATE RESPONSE OF SOME BIOLOGICAL SAMPLES BEFORE AND AFTER DEPROTEINIZATION WITH VARIOUS AGENTS

| Deproteinization agent                            | Amount measured as $SCN^-$ (ppm) |        |       |
|---|----------------------------------|--------|-------|
|   | Skim milk                        | Plasma | Urine |
| —   | 112                              | 130    | 9.8   |
| Methanol (4:1, v/v)                               | 1.8                              | 2.1    | 5.8   |
| Picric acid (4:1, v/v)                            | 2.2                              | 3.3    | 10.0  |
| ZnSO <sub>4</sub> -Ba(OH) <sub>2</sub> (4:1, v/v) | 28.9                             | 43.9   | 8.5   |
| Perchloric acid (0.6 N)                           | 14.5                             | 5.3    | 9.2   |

obtained (Table III), it is unlikely that a product other than cyanogen bromide would be formed as it behaved similarly in GSC and in the reaction with pyridine and *p*-phenylenediamine<sup>12</sup>.

The origin of the cyanogen bromide formed is not well understood. Plimmer<sup>21</sup> found that oxidation of proteins with chromic acid or Neumann's nitric acid mixture yielded HCN. The reported amounts of hydrogen cyanide evolved after oxidation of proteins are of the same magnitude as we found following direct bromine treatment (Table III). Therefore, accurate results cannot be obtained without careful deproteinization of biological material. Soluble protein fractions, such as are probably present in waste water, interfere when the method of Nota and Palombi<sup>19</sup> is used without the deproteinization step.

TABLE III

GAS CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC THIOCYANATE RESPONSES ON PROTEIN SOLUTIONS

Pure protein (2 mg) was dissolved in 1 ml of 0.5 M NaCl. Urine, plasma and skim milk samples were diluted 5-fold with water; 2 g of meat was homogenized with 10 ml of 0.5 M NaCl, centrifuged and filtered. Bromine treatment was performed on these solutions as described.

| Sample               | Response (ppm of thiocyanate) |                    |
|----------------------|-------------------------------|--------------------|
|                      | GSC                           | Spectrophotometric |
| Cytochrome C         | 3520                          | 3550               |
| N,N-Dimethylcasein   | 2340                          | 2000               |
| Bovine serum albumin | 1620                          | 2160               |
| $\gamma$ -Globulin   | 1550                          | 2450               |
| Skim milk            | 112                           | 82                 |
| Whole plasma         | 130                           | 96                 |
| Meat extract         | 11.5                          | 14.5               |
| Urine                | 9.8                           | 16                 |

#### *Selection of a deproteinization agent*

Several deproteinization agents compatible with the procedure were tried on the same samples. Comparison of the results (Table II) indicated that methanol and picric acid were particularly effective in removing proteins. However, the picric acid used contained impurities, resulting in peaks with long retention times in GSC and

requiring long time intervals (*ca.* 30 min) between consecutive injections. Deproteinization with perchloric acid failed with urine and yielded higher thiocyanate levels for skim milk and plasma. The higher results could be due to incomplete removal of protein or to a release of protein-bound thiocyanate under the influence of the more strongly bound perchlorate ion<sup>22</sup>.

Deproteinization of urine with methanol and determination of thiocyanate were compared with a spectrophotometric method based on a modification of the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  method<sup>6</sup>. The results obtained agreed closely (Table IV).

TABLE IV

COMPARISON BETWEEN THE GAS CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC DETERMINATION OF THIOCYANATE IN URINE

| Sample No. | Amount of $\text{SCN}^-$ (ppm) |                   |
|------------|--------------------------------|-------------------|
|            | GSC                            | Spectrophotometry |
| 1          | 6.9                            | 5.7               |
| 2          | 10.6                           | 10.0              |
| 3          | 6.7                            | 6.2               |
| 4          | 3.7                            | 4.1               |
| 5          | 5.0                            | 5.4               |
| 6          | 11.7                           | 11.8              |

#### *Reproducibility of the GSC procedure*

The reproducibility of the GSC procedure, was tested on different samples. Table V indicates that the thiocyanate level in different biological samples can be determined to within  $\pm 5\%$ .

TABLE V

REPRODUCIBILITY OF THE GSC PROCEDURE ON VARIOUS SAMPLES

| Sample | Number of determinations | Mean value $\pm$ standard deviation | Coefficient of variation (%) |
|--------|--------------------------|-------------------------------------|------------------------------|
| Meat   | 12                       | $3.5 \pm 0.2$                       | 5.7                          |
| Liver  | 9                        | $4.26 \pm 0.32$                     | 7.5                          |
| Kidney | 5                        | $5.95 \pm 0.23$                     | 3.8                          |
| Plasma | 6                        | $4.69 \pm 0.21$                     | 4.5                          |
| Urine  | 15                       | $28.14 \pm 1.07$                    | 3.8                          |
| Milk   | 4                        | $2.34 \pm 0.1$                      | 4.5                          |

After adding known amounts of thiocyanate to urine, plasma, milk and meat extracts, 90–100% of the thiocyanate added was recovered (Table VI).

#### *Determination of thiocyanate in urine after administration of $\text{KS}^{14}\text{CN}$ to rats*

The continuous administration of  $\text{KS}^{14}\text{CN}$  to rats over prolonged periods results in a "steady-state" condition in which the specific activity of  $\text{SCN}^-$  in the urine is identical with that of the ingested thiocyanate<sup>23</sup>. The thiocyanate level in urine, determined by GSC, can then be compared with that found by isotope dilution analysis.

TABLE VI

RECOVERY OF THIOCYANATE IN BIOLOGICAL SAMPLES FOLLOWING DEPROTEINIZATION WITH METHANOL

| Sample                         | Concentration of $\text{SCN}^-$ (ppm) |                |              |
|--------------------------------|---------------------------------------|----------------|--------------|
|                                | Before addition                       | After addition | Recovery (%) |
| Urine                          | 15.2                                  | 20.5           | 106          |
|                                | 5.1                                   | 9.6            | 90           |
| Plasma                         | 2.0                                   | 6.4            | 88           |
| Meat                           | 6.4                                   | 11.5           | 102          |
|                                | 4.5                                   | 28.2           | 95           |
| Milk                           | 2.0                                   | 6.1            | 82           |
| Mean $\pm$ S.D. : $95 \pm 8.4$ |                                       |                |              |

In preliminary experiments, two female Wistar rats were given drinking water containing 74 or 37 ppm of thiocyanate for 8 days. The 24-h urine was collected, deproteinized with methanol and analysed by the GSC method. The results are shown in Fig. 3. The daily excretion of thiocyanate in urine approached constant levels after 3 days of thiocyanate treatment. It was calculated that 75% of the thiocyanate ingested was excreted in the urine. Following withdrawal of thiocyanate, the urine levels returned to normal within 2 days.

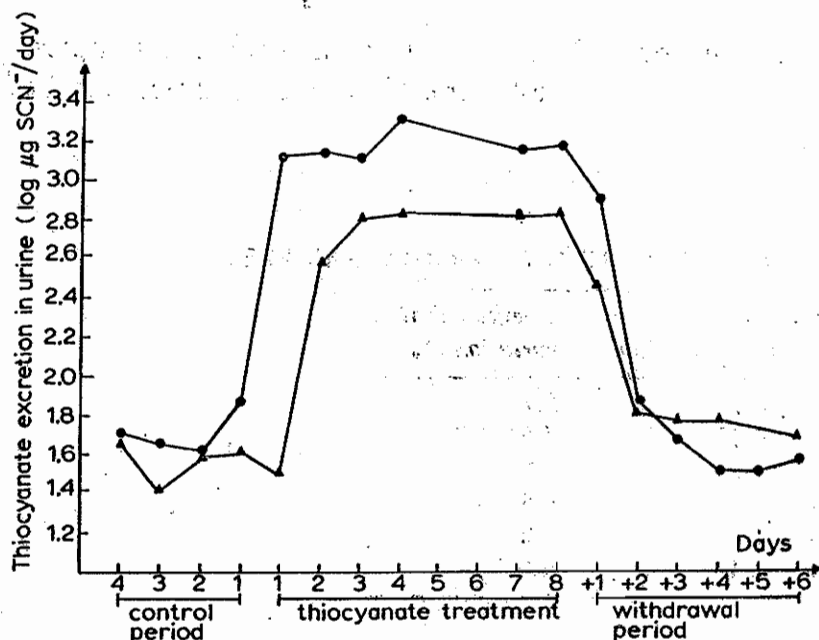


Fig. 3. Daily total urinary thiocyanate excretion by rats after chronic oral intake of 74 ppm (●) or 37 ppm (▲) of  $\text{SCN}^-$  in the drinking water. Volume ingested, *ca.* 30 ml/day; volume of urine, 5–10 ml/day.

#### *Determination of thiocyanate in biological fluids and tissues after oral administration of thiocyanate*

Two female Wistar rats, of the same weight and age, were placed in separate metabolism cages and maintained on the same stock pellets. The drinking water of one rat was replaced with a solution containing 0.2 mg/ml of  $\text{KS}^{14}\text{CN}$  (specific



activity, 12.5 cpm per milligram of  $S^{14}CN^-$ ) and 5 mg/ml of sodium chloride. After 3 days, cardiac puncture was performed under ether anaesthesia. The animals were killed by opening the chest and the liver, kidney and portions of some muscles were removed. All samples were immediately frozen at  $-25^\circ$  and stored until analysed.

The plasma, tissues, drinking water and urine were homogenized, deproteinized with methanol and analysed by GSC.

Table VII shows that the daily intake of 6 mg of KSCN results only in a 2–3-fold increase in the levels measured in plasma, kidney and liver as compared with a control rat. Ermans *et al.*<sup>23</sup> showed that blood thiocyanate levels in rats were not much affected by the dose when the compound was administered in the diet.

TABLE VII

THIOCYANATE LEVELS IN TISSUES AND BIOLOGICAL FLUIDS OF RATS AFTER DAILY ADMINISTRATION OF 0 (CONTROL) AND 6 mg OF  $KS^{14}CN$

| Sample | $SCN^-$ (ppm) |                      |
|--------|---------------|----------------------|
|        | Control rat   | $SCN^-$ -treated rat |
| Liver  | 2.4           | 7.0                  |
| Kidney | 4.3           | 10.3                 |
| Plasma | 6.7           | 16.7                 |
| Urine  | 5.8           | 154.0                |

In this experiment, the total radioactivity of the samples was determined by solubilization of a weighed amount of tissue with Soluene. After measurement of the specific activity of the methanol extract, the extraction yields of  $^{14}C$  from the biological specimens were calculated. The results (Table VIII) show that the extraction of the  $^{14}C$ , present in tissue or body fluids, into methanol is virtually quantitative. After treatment of the extract with bromine, *ca.* 75% of the  $^{14}C$  activity of the methanol extract is extracted into the benzene layer. As the extraction yields are similar to those

TABLE VIII

COMPARISON OF GAS CHROMATOGRAPHIC AND RADIOACTIVE  $SCN^-$  DETERMINATIONS IN TISSUE AND BODY FLUID EXTRACTS OF A  $KS^{14}CN$ -TREATED RAT

| Sample                   | Specific activity<br>( $10^{-3}$ cpm/g) |         | Extraction yield<br>of $^{14}C$ (%) |      | $SCN^-$ (ppm) |                  | Specific activity<br>( $10^{-6}$ cpm/mg $SCN^-$ ) |
|--------------------------|---|---------|-------------------------------------|------|---------------|------------------|---|
|                          | Tissue                                  | Extract | Tissue                              | BrCN | GSC           | Isotope dilution |   |
| Liver                    | 88                                      | 84      | 95                                  | 70   | 7.0           | 7.04             | 12.0  |
| Kidney                   | 109                                     | 106     | 97                                  | 75   | 10.3          | 8.70             | 10.3  |
| Right thigh              | 34                                      | 32      | 94                                  | 76   | 4.8           | 2.72             | 6.7   |
| <i>Longissimus dorsi</i> | 28                                      | 27      | 94                                  | 73   | 4.1           | 2.23             | 6.6   |
| Left thigh               | 29                                      | 30      | 105                                 | 75   | 4.4           | 2.31             | 6.8   |
| Drinking solution        | 1426                                    | —       | —                                   | 75   | 114.0         | 119.6*           | 12.5  |
| Plasma                   | 170                                     | 176     | 104                                 | 75   | 16.7          | 13.6             | 10.1  |
| Urine                    | 1580                                    | —       | —                                   | 75   | 154.0         | 126.3            | 10.2  |

\* Calculated from  $KS^{14}CN$  solution.

found after treatment of standard thiocyanate solutions with bromine (Table I), it is suggested that the extracted radioactivity is present in  $\text{Br}^{14}\text{CN}$ .

The specific activity in each sample was calculated by dividing the specific activity of each extract by amount of thiocyanate found by GSC. The results (Table VIII) indicate that the specific activities of thiocyanate in liver, kidney, plasma, urine and drinking solution are of the same magnitude. If isotopic steady-state conditions were reached, it was expected that the specific activities of thiocyanate in plasma and urine of rats would approach that of the thiocyanate drinking solution.

The nearly constant specific activities found in urine, plasma, kidney and liver indicate equilibrium of the thiocyanate in these extracts with ingested thiocyanate. From the ratio of the specific activity in the extract to the specific activity of thiocyanate in the drinking solution, the minimal amount of thiocyanate present in the tissues can be calculated (Table VIII). Comparison of the thiocyanate content calculated from isotope dilution with that determined by GSC indicates that the method described permits the accurate determination of this ion in plasma, urine, liver and kidney extracts.

The comparatively low specific activities of thiocyanate in meat extracts compared with that of the plasma was not expected. The lower specific activity could be due to a low exchange rate of meat-tissue thiocyanate with the plasma or, although less probable, to an endogenous dilution of the radioactive thiocyanate with inactive thiocyanate formed by the tissue from other non-radioactive precursors (e.g., cyanide)<sup>24</sup>. As thiocyanate tissue levels were low, it was decided to study the influence of a similar thiocyanate load on the thiocyanate content of the meat.

Perchloric acid deproteinization yielded high  $^{14}\text{C}$  recoveries from different tissues. However, specific activities in the perchloric acid extracts were three to six times lower than those obtained in the methanolic extracts. The higher thiocyanate levels measured in perchloric acid extracts may be ascribed to inadequate deproteinization.

#### *Thiocyanate content of muscles after treatment with thiocyanate*

The experiment was performed on four male Wistar rats: two rats were given normal drinking water (controls) and the other two were offered a drinking solution corresponding to 6 mg of KSCN per day. The animals were killed 49 days later and six muscles were dissected, cooled and analysed by GSC. From the results (Table IX), it is evident that treatment with relatively high thiocyanate levels does not result in a significant increase in the thiocyanate content of the muscles studied.

As the distribution of thiocyanate in ruminant tissues may differ from that of monogastric animals, a 27-kg male goat was given 300 mg of KSCN daily for 5 days, followed by 600 mg daily for 3 days, after which it was killed. Blood was taken from the *vena jugularis* during the course of the experiment. The plasma thiocyanate level increased from 2.1 ppm before the experiment to 3.7 ppm during the first 5 days and to 4.5 ppm on the eighth day. At this time the thiocyanate levels in liver, kidney, *longissimus dorsi*, *biceps femoris* and urine were 1.5, 2.7, 2.5, 2.3 and 80 ppm, respectively. These results are in contrast with those obtained on rats, where the concentration found in the liver and kidney were of approximately the same magnitude as that found in the plasma.

The results obtained on rats and goat clearly show that the administration of

TABLE IX

COMPARISON OF THIOCYANATE LEVELS IN MUSCLES OF CONTROL AND THIOCYANATE-TREATED RATS (6 mg/DAY FOR 49 DAYS)

| Muscle                      | Control rats |      | SCN <sup>-</sup> -treated rats |      |
|-----------------------------|--------------|------|--------------------------------|------|
|                             | 1            | 2    | 1                              | 2    |
| <i>Biceps femoris</i>       | 5.26         | 5.15 | 5.62                           | 5.51 |
| <i>M. gastrocnemius</i>     | 4.04         | 3.92 | 4.45                           | 5.0  |
| <i>Quadriceps femoris</i>   | 4.13         | 4.45 | 4.52                           | 4.96 |
| <i>Gluteus max. et min.</i> | 4.25         | 4.04 | 4.43                           | 4.58 |
| <i>M. long. dorsi</i>       | 4.51         | 4.43 | 4.53                           | 4.45 |
| <i>Iliopsoas</i>            | 4.14         | 3.75 | 5.40                           | 4.72 |

thiocyanate does not result in a significant increase in the thiocyanate level in different muscles.

#### CONCLUSION

The GSC method described permits the rapid, simple and accurate determination of thiocyanate in body fluids and tissues of animals. In comparison with spectrophotometric methods<sup>9-14</sup>, this GSC method proved to be less laborious for determining thiocyanate levels in plasma and milk. Moreover, the use of unstable or carcinogenic reagents is avoided.

The analysis of thiocyanate levels in meat and organs can be carried out, to our knowledge, only by using the GSC method described. However, high urinary levels can be determined by GSC as well as by the simple Fe<sup>2+</sup>/Fe<sup>3+</sup> spectrophotometric method (Table IV).

It was also observed that cyanogen bromide is formed during the reaction of proteins with bromine. Therefore, samples containing proteins (e.g., waste water) should be carefully deproteinized with a suitable reagent (e.g., hot methanol) before the application of a method based on BrCN determination.

As thiocyanate is a natural constituent of animals and plants, the GSC method was checked by comparison with the results obtained by isotope dilution after oral administration of KS<sup>14</sup>CN to rats. Administration of  $\pm 30$  mg of KSCN/day/kg to rats resulted in a 50- to 100-fold increase in the urinary SCN<sup>-</sup> level. Normal SCN<sup>-</sup> concentrations were restored within 3 days after administration ceased. Under thiocyanate treatment, the plasma and organ levels increased only 3- to 5-fold. Even prolonged administration of thiocyanate to rats and goats did not result in a significant increase in the meat SCN<sup>-</sup> levels.

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