liquid-liquid extraction; however, in the presence of strong complexing agents for tetravalent actinides, the reduction is very slow. A more detailed account of the experiments will soon be given.

1. Connick, R. E. *Ch. 8 of "The Actinide Elements", National Nuclear Energy Series IV-14A*, McGraw-Hill Book Co, New York 1954.
2. Culler, F. L. § 5—2 of *"Process Chemistry, Vol. I", Progress in Nuclear Chemistry, Series III*, Pergamon Press Ltd, London 1956.
3. Rydberg, J. and Bernström, B. *Acta Chem. Scand.* **11** (1956) 86.

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Tritium Labelling of *p*-Aminosalicylic Acid (PAS)

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For the study of the metabolic properties of the tuberculostatic agent *p*-aminosalicylic acid* (PAS) [1], it was of importance to obtain tritium labelled PAS. In order to avoid cumbersome syntheses, the possibility of using recoil tritons for the labelling [2] was investigated.

In this method the organic compound to be labelled is mixed with a lithium salt, and the mixture is irradiated with slow neutrons. From nuclear reactions between these neutrons and ^{6}Li, tritium atoms with a kinetic energy of 2.7 MeV** are produced. These fast tritons are successively slowed down by collisions with surrounding atoms, which cause heavy destruction along the path of the triton, until it is finally trapped in some molecule. Since the neutron irradiation usually is carried out in a nuclear reactor, considerable destruction of the organic compound is also caused by other high energy particles present (neutrons, γ-quanta, *etc.*). The irradiated mixture will therefore contain a number of decomposition products of the original compounds, which all may be labelled with tritons. As has been pointed out [3], the difficulty lies not in the labelling, but in the isolation of a pure product.

In our experiment, 6.0 g PAS was mixed with 0.6 g Li_2CO_3 and irradiated with a slow neutron flux of 1.4×10^{12} n/cm^2/sec for 120 h (temperature ~40° C) in the reactor at A. B. Atomenergi in Stockholm. The isolation of pure PAS from the product, which had slightly darkened during the irradiation, was made in the following manner.

The product was mixed with amyl alcohol, which dissolves PAS as well as some of the organic decomposition products but not the Li_2CO_3. After solid substances had been filtered off, the solution was shaken with an equal volume of 0.1 M $NaHCO_3$ in water.

The distribution ratio q of PAS between the two solvents is given by the equation

$$q = \frac{k_d}{1 + k_a [H^+]}$$

at pH > 3 (at lower pH another term has to be added in the denominator to correct for the amphoteric character of PAS). Since the dissociation constant [4] k_a of the carboxylic hydrogen in PAS is $10^{-4.08}$, and the distribution constant k_d of undissociated PAS between amyl alcohol and water was determined to be 36 ± 3, it is easily calculated that about 95 % of PAS goes down into the aqueous phase when this is 0.1 M in $NaHCO_3$ (pH ~ 7).

The aqueous solution thus obtained was washed five times with equal portions of amyl alcohol. Since the equation above is generally valid for monobasic acids, these washings with amyl alcohol remove all organic decomposition products with $k_a < 10^{-7}$ and $k_d > 1$ (including the probable decomposition product *m*-aminophenol (MAP) with [5] $k_a = 1.3 \times 10^{-10}$ and $k_d \sim 2$). The aqueous solution was then treated in two different ways.

In *procedure A*, 0.1 M HCl was added until pH 3.8 was reached. The solution was then shaken with an equal volume of amyl alcohol. From the equation above, it can be calculated that at pH 3.8 about 95 % of PAS will be obtained in the organic phase, while a number of organic impurities are left in the aqueous phase. By this procedure all substances are removed which do not have k_a and k_d close to that of PAS.

In *procedure B*, the aqueous phase was cooled in ice, and HCl was added until a

* Kindly supplied by A. B. Ferrosan, Malmö, Sweden.

** MeV = million electron volts.

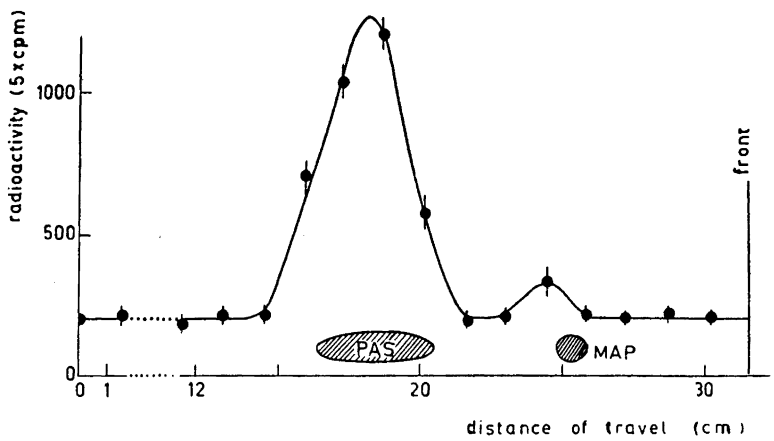

Fig. 1. Paper chromatogram of PAS purified according to method B. For comparison spots obtained with pure PAS and MAP are also shown.

pH around 3 was reached, when PAS precipitates. Now it is known that at this acidity PAS may decompose to MAP[6]; however, under the conditions used not more than a few percent was decomposed (see further below). The advantage of this procedure is that it leads to a solid PAS compound somewhat more rapidly than procedure A.

In both procedures tritium, which is unstably bound in the pH range 4—7 (as *e. g.* carboxylic hydrogen), is removed.

In order to test the purity of the products, paper chromatograms were run on Whatman No. 4 papers using a mixture of 40 % methyl alcohol, 20 % amyl alcohol, 20 % benzene, and 20 % water[7]. Due to the low energy of the β-radiation from tritium (0.018 MeV, corresponding to a maximum range of about 0.7 mg/cm^2), the radioactivity from tritium could not be measured directly on the paper chromatograms. These were therefore cut in pieces, and the activity eluted by several washings with the solvent mixture. The eluates were collected in aluminum dishes and evaporated, and the activity of the samples was measured in an internal proportional counter.

Fig. 1 shows the results obtained with PAS purified according to method B. As a comparison, parallel runs were made with ordinary PAS and MAP; these spots were determined either in ultraviolet light or developed with Ehrlich reagent. It is believed that the activity found is only due to tritium in PAS and MAP. The MAP may originate from the decomposition of some PAS at the high acidity used in the final step. By integrating the areas under the peaks in Fig. 1, after the back ground has been subtracted, it is found that 4 ± 2 % of the total tritium activity is due to tritium is bound in MAP; the rest of the activity to PAS.

The specific activity of the product was determined in the same counting arrangement, which had an efficiency of about 60 % for the β-radiation from tritium. This value includes geometry factors and back-scattering but excludes self-absorption. Since the self-absorption in the samples is very high, successive dilutions were made with ethyl alcohol, and the activity of aliquotes measured after each dilution. The following results were obtained:

μg PAS in sample	cpm	cpm/μg PAS
278	43 000	155
22.2	6 100	274
1.78	1 000$\pm$40	561
0.143	120$\pm$12	840
0.0114	11$\pm$5	970

The specific activity approaches a constant value, which is estimated to 1 500 ± 500 cpm/μg PAS. With this value and knowing the efficiency of the counter, the yield in the tritium labelling of PAS is calculated to 1.5 ± 0.5 mC/g PAS.

1. Hanngren, Å. *To be published.*
2. Wolfgang, R., Rowland, F. S. and Turton, C. N. *Science* **121** (1955) 715.
3. Rowland, F. S. and Wolfgang, R. *Nucleonics* **14** No. 8 (1956) 58.
4. Ågren, A. *Acta Chem. Scand.* **8** (1954) 1059.
5. Kuhn, R. and Wassermann, A. *Helv. Chim. Acta* **11** (1928) 23, 25.
6. Ågren, A. *Farmaceutisk Revy* **54** (1955) 225.
7. Ekman, B. *Acta Chem. Scand.* **2** (1948) 383.

Preparation of 2-Methyl[1-^{14}C]-octadecanoic Acid

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2-Methyloctadecanoic acid has been prepared by several investigators, *e. g.* by Schneider and Spielman [1] from diethyl methylmalonate and hexadecyliodide and by Morgan and Holmes [2] from methylmagnesiumiodide and 2-bromooctadecanoic acid.

For the preparation of [carboxy-^{14}C] 2-methyloctadecanoic acid a Kolbe electrolysis of hexadecanoic acid and sodium 3-[^{14}C]cyanobutyrate and subsequent hydrolysis of the cyano group seemed more suitable and was found to be convenient. The cyanobutyrate (previously prepared in a different way by Widequist [3]) was easily obtained in comparatively good yield from ethyl crotonate and sodium [^{14}C]cyanide (*cf.* the preparation of methylsuccinic acid by Higginbotham and Lapworth [4]).

Experimental. Sodium 3-[^{14}C]cyanobutyrate (I). A solution of ethyl crotonate (0.91 g) in ethanol (3.6 ml) was mixed with 0.41 g of sodium [^{14}C]cyanide dissolved in 1.0 ml of water and the mixture refluxed on a water bath for 5 h. The solvents were removed by distillation and the residue extracted with boiling acetone. The insoluble fraction was filtered off. On cooling the filtrate, colourless crystals of I (0.72 g, 67 %) separated. They were dried at 0.4 mm Hg, 78°. (Found: C 44.0; H 4.7; N 10.1. Calc. for $C_5H_6NO_2Na$: C 44.4; H 4.5; N 10.4.)

2-Methyl[1-^{14}C]octadecanoic acid (III). A solution of I (0.64 g of crude product) and hexadecanoic acid (7.0 g) in methanol (75 ml) was electrolysed between platinum electrodes as described by Stenhagen [5]. The precipitate (triacontane and a small amount of 2-methyloctadecanenitrile, II) was filtered off at room temperature and dissolved in boiling methanol. The solution was cooled to 20° and the precipitate again removed by filtration. The combined methanol solutions were evaporated and the residue extracted with ether, saturated with water. After removal of free acids from the ether solution by the ion exchanger Amberlite IRA 400, the ether was evaporated and the product hydrolysed by refluxing with ethanolic potassium hydroxide solution. Water was added to the hydrolysate, and hydrocarbons were removed by extraction with ligroin. Acidification of the alcohol-water layer and extraction of the 2-methyl[1-^{14}C]octadecanoic acid with ether, yielded 0.65 g (46 %) of crude III. After recrystallization from methanol and ligroin, 0.36 g of pure acid, m.p. 54.5—55.0°, remained. Schneider and Spielman [1] give m.p. 54.5°, Cason, Wolfhagen, Tarpey and Adams [6] 54.6—55.1° and Morgan and Holmes 58°.

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$$CH_3CH{=}CHCOOC_2H_5 + Na^{14}CN + H_2O \rightarrow \underset{\text{I}}{CH_3CH(^{14}CN)CH_2COONa} + C_2H_5OH.$$

$$CH_3(CH_2)_{14}COOH + CH_3CH(^{14}CN)CH_2COONa \xrightarrow[\text{in } CH_3OH]{\text{electrolysis}}$$

$$CH_3(CH_2)_{15}CH(CH_3)^{14}CN \xrightarrow{\text{hydrolysis}} CH_3(CH_2)_{15}CH(CH_3)^{14}COOH.$$

II III