274. *Radioactive Sulphur for Biochemical Experiments.*

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In order to determine whether radioactive sulphur in organic thio-compounds could be used in biological investigations, experiments were made to discover whether the sulphur exchanged with radioactive sulphur as H_2S, S'', and SH' in aqueous solution. Although 5% of exchange could have been detected, none was observed.

The choice of a radioactive indicator in the study of such physiologically important compounds as amino-acids and proteins seems to be restricted to sulphur, since it is the only constituent element having a radioactive isotope of reasonably long half-life (^{35}S, 88 days, Buch and Anderson, *Z. physikal. Chem.*, 1936, *B*, **32**, 238).

Further, it seems likely that the very soft β-radiation from ^{35}S of upper limit 125 kV (Libby, *J. Amer. Chem. Soc.*, 1937, **59**, 2474) may result in a novel highly local effect in therapeutic application. The rise in glutathione content of cancer tumour coincident with its fall in the rest of the body (Voegtlin and Thompson, *J. Biol. Chem.*, 1926, **70**, 801), and the abnormally low disulphide-group concentrations in cancer serum observed by Brdicka and his co-workers (Brdička, *Nature*, 1937, **139**, 330; Bergh, Henriques, and Schousboe, *ibid.*, 1938, **141**, 751) point to interesting curative possibilities, which are being investigated by Leo Szilard.

All the above uses require that the radioactive sulphur shall not exchange between the labelled molecule and neighbouring sulphur atoms. Such an exchange would result from electrolytic dissociation, or from such process as attack and substitution by a negative ion (negative mechanism), *i.e.*, $\overset{-}{\mathrm{H}}\mathrm{S} + \mathrm{R{\cdot}S^*H} \rightarrow \mathrm{H{\cdot}SR}, + \overset{-}{\mathrm{S}}{}^*\mathrm{H}$ and the nitroprusside test in mildly alkaline solution showed that ionisation of the SH group was a possibility.

Of the biologically important compounds, cysteine, cystine, glutathione, methionine, and the proteins, the first forms a convenient representative, since it is structurally related to all of them. Hence, if its SH group were found to be non-labile, the same would apply to the sulphydril groups of the above class, and still more strongly to the disulphide linkages. The first experiments were therefore made with cysteine.

The ^{35}S was made by the neutron–proton reaction from chlorine as carbon tetrachloride containing a trace of sulphur as carrier. After alkaline oxidation of the product, the aqueous layer was treated by the repeated addition of phosphate and precipitation of silver phosphate in order to remove the ^{32}P formed by the (n; α) reaction. The radioactive sulphur was then precipitated as barium sulphate. Much care was taken to ensure the complete removal of ^{32}P, as its activity is initially much greater than that of ^{35}S, and it was readily co-precipitated with barium sulphate. Precipitates so contaminated lost radioactive phosphate by exchange in a boiling solution of sodium phosphate only very slowly.

The activity of ^{35}S is not detected by a normal β-ray counter having 0·1-mm. aluminium walls, and the counter used had walls of 0·008-mm. aluminium leaf with a movable source all inside a low-pressure envelope arranged in a manner similar to Libby's screen wall counter (*Physical Rev.*, 1934, **46**, 196).

The yield of ^{35}S is high; for instance, a single 500 mC radon–beryllium source used for 12 days at the centre of a 1-litre flask of carbon tetrachloride produced an amount of ^{35}S having an activity of 2000 impulses/min. The source used in this and all other measurements had a thickness corresponding to 3 mg./cm.^{2} of barium sulphate, so there was some self absorption of the softer β-radiation.

The activities observed in ^{35}S and ^{32}P after a short irradiation were approximately inversely proportional to their half-lives, so, allowance being made for self-absorption of the β-radiation from ^{35}S, it was formed at a greater rate than ^{32}P under the conditions of irradiation given above.

The following is a description of a characteristic experiment. A portion of barium sulphate containing radio-active sulphur was weighed, and the activity of a known fraction of it measured. The barium sulphate was then heated with sugar carbon for 10 hours. The resulting barium sulphide was decomposed by hot aqueous trichloroacetic acid, and the hydrogen sulphide passed through water, calcium chloride, and phosphoric oxide, and condensed in liquid air. The purified gas obtained by distillation was passed into an evacuated bulb containing air-free water (20 c.c.) and 84 mg. of cysteine hydrochloride. After 6 hours' heating in a steam-jacket with constant shaking, the hydrogen sulphide was pumped off. This and the cysteine hydrochloride were then separately oxidised, and sulphur precipitated as barium sulphate. Samples of these were then measured for radioactivity:

	Wt., g.	Activity after deduction of background (29·5).
Initial $BaSO_4$	0·361	—
Measuring sample of above	0·122	101 ± 2·5
$BaSO_4$ recovered from H_2S	0·309	—
Measuring sample of above	0·106	90 ± 3
Cysteine hydrochloride	0·084	—
$BaSO_4$ from cysteine hydrochloride ...	0·116	0 ± 1·5

In order to test further the stability of the cysteine molecule, experiments were done under various conditions. In the last experiment the increased concentration of the HS′ ions would have given an increased velocity of exchange had a negative mechanism for exchange existed: no exchange was observed even at 100° although 5% could have been detected.

	Time, hrs.	Temp.
Cysteine hydrochloride + H_2O + H_2S	20	20°
Cysteine hydrochloride + H_2O + H_2S	6	100
Cysteine + H_2O + NaSH + H_2S	6	20

It may be concluded, therefore, that the thiol group in cysteine and allied compounds is very firmly bound, and that radioactive sulphur may be used as a satisfactory indicator in such compounds. It also follows that the nitroprusside reaction with sulphydril is not an ionic one.

Further experiments were carried out at 100° for 6 hrs. on the following organic thio-compounds :

Thioglycollic acid + H_2O + H_2S
Allylthiourea + H_2O + H_2S
Thiourea + H_2O + H_2S
Thiourea + H_2O + NaSH + H_2S
Thiourea + H_2O + NaSH + H_2S + $Cu^{\cdot\cdot}$ + $Fe^{\cdot\cdot}$ + $Mn^{\cdot\cdot}$

No exchange was observed, even in the presence of the above-mentioned metal ions which are known to catalyse reactions involving organic thio-compounds (Warburg and Sakuma, *Arch. ges. Physiol.*, 1923, **200**, 203; Baur and Preis, *Z. physikal. Chem.*, 1936, *B*, **32**, 65; Goldberg, *Ber.*, 1904, **37**, 4526).

The above work has had some confirmation by the recent biological synthesis of glutathione containing radioactive sulphur (Franklin, *J. Franklin Inst.*, 1939, p. 722).

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