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Utilization of Radiosulphate by Higher Plant Tissues

THERE is now considerable information concerning the pathway by which sulphate is incorporated into the amino-acids in micro-organisms[1]. In contrast, very little is known about the route for incorporation of sulphate into the amino-acids of higher plants or of the interconversions of these sulphur-containing amino-acids[2].

In the work recorded here, a variety of higher plant materials were examined for the incorporation of radiosulphate into the amino-acid fraction. In particular, the leaves, hypocotyls and cotyledons of radish seedlings were found to show a rapid incorporation of radiosulphate into the amino-acids. Similar patterns of incorporation were found when using cell-free extracts of these tissues supplied with adenosine triphosphate.

In the experiment shown in Table 1, 10-day-old radish seedlings were severed at soil-level and the hypocotyls placed in the buffered radiosulphate solutions as indicated. Samples of the seedlings were placed in the dark and in the light as shown and the uptake and incorporation of radiosulphate into the amino-acids were followed over periods up to 1 h.

At the end of the experimental periods, the seedlings were killed in boiling 80 per cent ethanol, and following homogenization, were extracted using dilute ethanol solutions and water[3]. Sulphate was removed from the soluble fraction by passing through 'Rexyn' columns in the hydrogen form and by ion exchange paper chromatography. The amino-acid fraction, eluted from the 'Rexyn' columns using 2 N ammonium hydroxide, was separated by two-dimensional paper chromatography[3]. The radioactive areas, ascertained by autoradiography, were eluted and the radioactivity assayed using a 'Mylar' window continuous gas flow Geiger–Müller tube. The counts were corrected for background and half-life decay.

It is evident from Table 1 that the uptake of radiosulphate was not appreciably affected by illuminating the seedlings. The rate of accumulation of sulphur-35 by the tissues was approximately linear over the 60 min experimental period. Activity was detected in the amino-acids and in material soluble in anhydrous ether. The latter fraction would presumably contain mustard oils which are abundant in plants related to the radish[4].

After 15 min of sulphate uptake in light or darkness, radioactivity was detected in the cysteine–cysteic acid area on the chromatograms. After 30 min, taurine-^{35}S was present in both treatments and methionine was labelled in the illuminated tissues. With increase in the experimental period to 1 h, the radioactivity in taurine decreased in the light but further increased in the seedlings in the dark. Furthermore, after 1 h, methionine sulphone-^{35}S was present in the tissues, the radioactivity

Table 1. UPTAKE AND INCORPORATION OF RADIOSULPHATE BY EXCISED RADISH SEEDLINGS IN LIGHT AND DARK (EXPRESSED AS COUNTS/MIN)

Fraction	Light*			Dark†		
	15 min	30 min	60 min	15 min	30 min	60 min
Total ^{35}S taken up	115,500	234,000	402,500	106,000	183,000	390,000
Ether solubles	—	550	1,850	—	1,000	900
Amino-acid fraction, cysteic acid and cysteine	360	640	680	1,740	1,920	2,360
Taurine	—	3,240	1,920	—	600	2,100
Methionine	—	200	1,000	—	—	500
Methionine sulphone	—	—	1,450	—	—	400

5 μc. of carrier-free $^{35}SO_4$ in 0·02 M *tris* buffer *p*H 7·4 was fed through the hypocotyls of 0·75-g samples of seedlings.
* Illuminated with 500 ft.-candles in moving air at 25° C.
† Incubated in still air at 25° C.

in this compound being greater under conditions of illumination. The results are therefore in agreement with an earlier report[5], where excised mung bean leaves produced cysteine and methionine when infiltrated with radiosulphate.

Similar results were obtained when radiosulphate solutions were fed to slices of radish cotyledons, slices of young radish leaves and intact radish leaves. Tomato and *Coleus* leaves readily produced cysteine-^{35}S from radiosulphate, but methionine-^{35}S was not detected even after a 3-h experimental period.

Cell-free extracts of radish leaves prepared in 0·5 M sucrose, 0·02 M magnesium chloride and 0·025 M *tris* buffer at *p*H 7·4, containing chloroplasts and mitochondria, incorporated $^{35}SO_4$ into the amino-acid fraction.

This incorporation was not markedly stimulated by additions of TPN or by a system producing reduced TPN. Additions of ATP (1 micromole), however, caused a five-fold increase in the sulphur-35 content of the amino-acid fraction as compared to the extracts where cofactor additions were not made. More detailed experiments on sulphate metabolism *in vivo* and *in vitro* by the tissues are now in progress.

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BIOLOGY

Two Components in the Development of Ray Cataract in Frogs

RECENTLY it has been shown in our laboratory that any intra-ocular operation on irradiated tadpoles seemed to result in the development of a ray cataract[1]. Later on, the same results were obtained in adult frogs and mice as well, in which an accelerated development of the aforementioned disease was also brought about by a fine pricking of the irradiated lens[2–4]. At the same time, judging by long-term observations on irradiated tadpoles and frogs, even a very heavy irradiation without subsequent operative intervention affects neither the transparency nor the structure of lenses. The experiments described here suggested to us that a direct relation existed between the degree of irradiation of the eye prior to operation and completeness of the opacity of the lens. Therefore, the question arose as to which doses of ionizing radiation—extreme, minimal, and maximal, combined with operative interference—provoked an accelerated development of the ray cataract.