an Rf value of 50, compared to GABA which had an Rf value of 45. In the high-voltage electrophoresis system, DAVA had a mobility equal to that of glycine, whereas GABA migrated halfway between glycine and lycine. By ion-exchange chromatography using a Technicon amino acid analyzer, GABA was eluted after 8 h and 40 min while DAVA was eluted after 10 h and 10 min. In all of the samples the latter appeared as a very sizable peak between ammonia and ornithine. Five saliva samples contained an average of 0.131 μmoles of DAVA/ml. This was equaled only by the amino acid proline and exceeded the quantity of any other amino acid in saliva. Average quantities of all of the free amino acids are listed in the Table. The portions of saliva which had been kept at room temperature for 2 days contained much more DAVA and proline than did the specimens chromatographed immediately after expectoration. This suggests that bacterial contamination and the process of putrefaction may be largely responsible for the presence of free DAVA and proline in human saliva, as had been suspected by FOSDICK and PIEZ[6]. Our experience indicates that meaningful biochemical data on human saliva must be obtained from specimens collected by sterile catheterization of the salivary gland ducts. Finally, we must conclude that GABA is not present in human saliva[8].

Quantitative values of free amino acids in human saliva as determined by ion-exchange chromatography

Amino acid	Average of 5 samples (μmoles/ml)	Amino acid	Average of 5 samples (μmoles/ml)
Taurine	0.071	α-amino butyric acid	trace
Aspartic acid	0.026	Valine	0.080
Threonine	0.005	Isoleucine	0.006
Serine	0.005	Leucine	0.008
Glutamine	0.006	Tyrosine	0.015
Proline	0.088	Phenylalanine	0.012
Glutamic acid	0.037	Lysine	0.032
Citrulline	0.013	Histidine	0.018
Glycine	0.144	Arginine	0.010
Alanine	0.032	δ-amino valeric acid	0.131

Résumé. Dans les travaux de divers laboratoires on peut lire que l'acide γ-amino-butyrique (GABA) existe dans la salive humaine. Nos récents examens chromatographiques ont mis en évidence que cet acide avait été identifié par erreur et qu'il représente en fait l'acide δ-amino-valérique (DAVA), un constituant de la salive provenant de l'action bacterienne.

P. M. DREYFUS, H. L. LEVY and MARY L. EFRON †

Department of Neurology, Massachusetts General Hospital, and the Joseph P. Kennedy, Jr. Memorial Laboratories, Massachusetts General Hospital, Boston (Massachusetts 02114, USA), 13 December 1967.

[8] This investigation was supported by research grants No. B-1906, NB-05096 and 1 FZHD-32,137 from the U.S. Public Health Service, National Institutes of Health.

The Influence of Lethal X-Irradiation on Trace Metal Uptake by the Mitochondrion

Marked variation has been demonstrated in the concentrations of trace elements in tissues of lethally X-irradiated rats (HEGGEN, OLSON, EDWARDS, CLARK and MAISEL[1]). Determinations of the post-irradiation dynamic picture of trace metal levels in a small intracellular functional entity, the mitochondrion, was undertaken in the present study by means of radioisotopic tracers. The spleen, one of the more radiosensitive tissues, was chosen as a source of mitochondria. The trace metals selected for study (zinc, manganese, cobalt, iron, chromium, selenium, and nickel) are coenzymatic in nature (DIXON and WEBB[2]).

Material and methods. The principles of laboratory animal care as promulgated by the National Society of Medical Research were observed.

Adult female albino rats were fed standard laboratory food ab libitum. Irradiation was carried out with a 300 kVP X-ray therapy unit, 900 rads total body (300 kV, 20 mA), target distance 85 cm, at a rate of 49.13 r/min, inherent filtration 4.75 mm Be, added filtration 2.0 mm Cu. One, three, and six days post-irradiation, 4 rats were sacrificed by neck fracture; their spleens were immediately removed and processed to isolate mitochondria (CLELAND and SLATER[3]). One washing with a solution consisting of sucrose (0.05 *M*), KCl (0.02 *M*), and Sorensen phosphate buffer, pH 7.45 (0.02 *M*) was performed. The mitochondrial pellet was resuspended in the sucrose-KCl-buffer solution and distributed among several polypropylene centrifuge tubes; about 0.2 mg nitrogen equivalent of protein per tube. 1 μc of carrier-free radioisotope (^{58}Co, ^{63}Ni, ^{51}Cr, ^{59}Fe, ^{54}Mn, ^{63}N, ^{75}Se or ^{65}Zn) in sucrose-KCl-buffer solution, was added to each tube (final volume 2 ml) and incubated at 4 °C for 1 h (SPECTOR[4]). Analyses were performed in triplicate. After centrifugation at 8000 *g*, the mitochondria were washed once with 10 ml of 0.001 *M* non-radioactive isotope in isotonic saline, recentrifuged, and transferred to screw-capped test tubes. Mitochondria from control (non-irradiated) rats were carried through the same procedure concurrently. Results were expressed in terms of c.p.m. of each specimen divided by the product of mg nitrogen (microkjeldahl assay) and c.p.m. of standard radioisotope (exactly $^1/_{100}$ of the amount added to the mitochondria). This expression standardizes amount of mitochondria, amount of radioactivity, and decay of the radioisotope in all specimens (ANDERSON[5]).

Results. The mean results of the experiment are presented in Table I. The pattern of change in cation uptake

[1] G. E. HEGGEN, K. B. OLSON, C. E. EDWARDS, L. B. CLARK and M. MAISEL, Radiat. Res. *9*, 285 (1958).
[2] M. DIXON and E. C. WEBB, *Enzymes* (Academic Press, New York 1958).
[3] K. W. CLELAND and E. C. SLATER, Biochem. J. *53*, 547 (1954).
[4] W. G. SPECTOR, Proc. R. Soc., Series B *141*, 268 (1953).
[5] J. E. ANDERSON, personal communication.

Table I. Trace metal uptake by splenic mitochondria

	Pre-irradiation	Post-irradiation Day 1	Day 3	Day 6
	cpm/mgN × cpm_h			
Iron	4.2	15.0	6.4	6.2
Zinc	2.3	3.7	1.4	1.5
Cobalt	1.6	2.3	1.3	0.9
Nickel	3.4	5.5	5.4	6.4
Selenium	14.0	12.0	15.0	25.0
Manganese	16.0	6.7	1.7	7.0
Chromium	29.0	23.0	21.0	36.0
	% of control			
Iron	100	360	150	150
Zinc	100	160	61	65
Cobalt	100	140	81	56
Nickel	100	160	160	190
Selenium	100	86	110	180
Manganese	100	42	11	44
Chromium	100	79	72	120

Table II.

	Iron (% of control)					
	Pre-irradiation	Post-irradiation Day 1	Day 2	Day 3	Day 4	Day 6
Content	100		200		340	390
Uptake	100	360		150		150

Table III.

	Zinc (% of control)					
	Pre-irradiation	Post-irradiation Day 1	Day 2	Day 3	Day 4	Day 6
Content	100		190		130	160
Uptake	100	160		61		65

Table IV.

	Manganese (% of control)					
	Pre-irradiation	Post-irradiation Day 1	Day 2	Day 3	Day 4	Day 6
Content	100		220		240	240
Uptake	100	42		11		44

appeared to be somewhat complex; increased uptakes of zinc, cobalt, iron, nickel and selenium, and decreased uptake of manganese and chromium seem to be the general trends. Deviation of each replicate from the mean was less than 10%. Repeats of several portions of the experiment at a later time gave values in the same range. Values obtained from control animals did not vary significantly over the 6-day period ($P < 0.05$).

Discussion. It is interesting to compare the results of this uptake study with data obtained under similar conditions of irradiation (HEGGEN et al.[1]). Although HEGGEN's group measured the content of the trace metals in whole tissues and this study measured the ability of mitochondria to take up the metals, some valid comparisons may be made since the site of action of these cofactor cations may be primarily intramitochondrial. It is possible, of course, that the uptake of metals from solution may only reflect diffusion or surface absorption and not intramitochondrial transport. Tables II, III and IV compare cation content (HEGGEN's study) with cation uptake (present study). A twofold increase in iron content was noted by the second day post-irradiation (Table II). The uptake study indicated nearly a fourfold increase in isotope exchange. A continual increase in iron content to about 5 times that of control was shown by the sixth day. The uptake, on the other hand, decreased to near control level by this time. These data appear to show an increased influx of iron with a concomitant decrease in efflux. The zinc content and uptake (Table III) were not quite double the control levels on the first and second days post-irradiation; by the sixth day, the uptake had decreased considerably. Here, the lower-than-control uptake value seems to indicate maintenance of a constant zinc content by the mitochondrion since the zinc content at the eighth day was very nearly the same as on the fourth and sixth days. The influx-efflux ratio in this case appeared to be nearly unity. Manganese content (Table IV) was double that of control on the second day post-irradiation and remained essentially the same until the eighth day, when it was 4 times that of control. Initially, the uptake decreased markedly, returning to the first day's level on the sixth day. These data indicate that manganese appears to be conserved within the mitochondrion solely by a diminution of efflux rather than by influx of ions, as in the cases of iron and zinc.

Uptake data for chromium (first and third days post-irradiation) followed those of manganese, while cobalt, nickel, and selenium uptakes were more akin to those of iron and zinc.

The preceding considerations indicate that the transport of certain trace metals across the mitochondrial membrane may be markedly altered by ionizing radiation. Although few early significant changes in enzyme activity after ionizing radiation have been demonstrated to date, further studies involving trace element cofactors may prove valuable in locating such biochemical lesions.

Zusammenfassung. Die Aufnahme von Spurenmetallen durch Mitochondrien der Milz von Ratten, die letalen Dosen von Röntgenstrahlen ausgesetzt worden waren, wurde in vitro durch radioaktive Tracerisotope bestimmt. Kurz nach der Bestrahlung wurde bei Kobalt, Eisen, Nickel, Selen und Zink eine erhöhte, bei Chrom und Mangan eine verringerte Aufnahme beobachtet.

W. J. DECKER

Research and Development Service, William Beaumont General Hospital, El Paso (Texas 79920, USA), 27 November 1967.