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Biological Studies with Arsenic^{76}. I. Preparation of Arsenic^{76} by Pile Irradiation of Cacodylic Acid.

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(Introduced by L. O. Jacobson.)

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Preliminary investigations into the production of radioactive arsenic, for distribution studies in animals and for clinical trial, indicated the need for inorganic radioactive arsenical compounds of a higher specific activity than could be obtained by pile irradiation of As_2O_3. This led to the consideration of the "Szilard Chalmers reaction."[1] Basically, this is a general reaction in which the recoil energy, imparted to the As nucleus upon neutron capture, ruptures the chemical bonds which hold it in the complex molecule, leaving it free and radioactive. D'Agostino[2] was able to separate arsenic^{76} from cacodylic acid after bombardment by a radium-beryllium source, and Szilard[3] stated that pile irradiation of cacodylic acid would give good yields of radioactive arsenic. Preliminary experiments confirmed this.[4]

Methods. Cacodylic acid $(CH_3)_2AsOOH$, is a colorless solid that is easily soluble in water. It melts at 200°C and is extremely stable, being unaffected by fuming nitric acid, aqua regia, or potassium permanganate.[5]

An accurately weighed quantity (300-750 mg) of this substance, finely powdered, is introduced into a soft glass vial (8 x 40 mm) that has a small glass eyelet fused to its rounded bottom. The open end of the vial is then sealed with paraffin and, eyelet end up, placed within a larger quartz tube (12 x 125 mm). The latter is evacuated, sealed, and irradiated in a neutron pile.

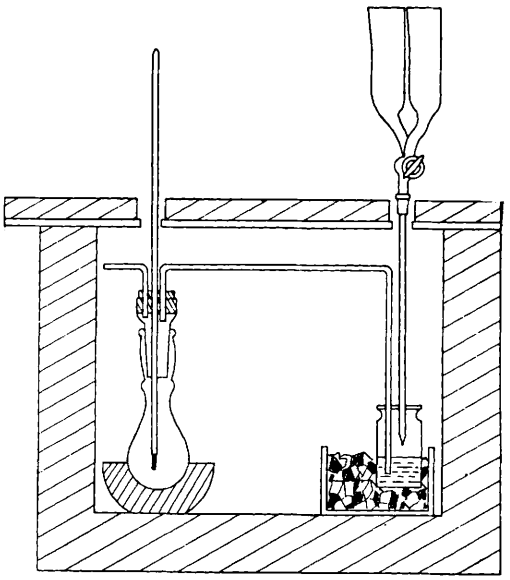

Fig. 1.
Cross section of lead shield with distilling apparatus in place.

Following irradiation, the quartz tube and its container are handled only by remote control apparatus and behind 10-15 cm of solid lead shielding. All chemical operations are carried on behind a U-shaped shield, having a floor and roof constructed of dovetailed, 10-cm thick, lead bricks, laid in alternating courses. A mirror, set at an angle at the open back of the shield, allows the operator to view the interior and the progress of the experiment. A cross section of the shield and the distilling apparatus it contains are shown in Fig. 1.

The irradiated quartz tube is slipped into a lead block placed behind the shield. The neck of the tube is then shattered by the remote control ratchet mechanism illustrated

1 Szilard, L., and Chalmers, P., *Nature*, 1934, **132**, 462.

2 D'Agostino, O., *Gazzetta Chimica Italiana*, 1935, **65**, 1075.

3 Szilard, L., personal communication.

4 Brues, A. M., ed., Quarterly Report, Biology Division, Argonne National Laboratory, August 1, 1947, ANL-4078.

5 Raiziss, G. W., and Gavron, J. L., *Organic Arsenical Compounds*, Chemical Catalog Co., Inc., New York, 1923.

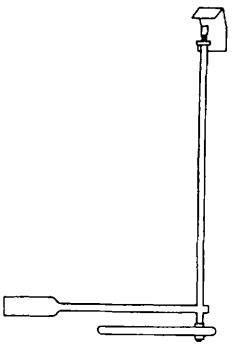

FIG. 2.
Remote control ratchet mechanism used in crushing the quartz capsule.

in Fig. 2. The "hot" cacodylic acid is transferred to the distilling flask with the aid of a right-angle hook that engages the eyelet on the bottom of the soft glass vial in which the acid is contained. The distilling vessel is an ordinary 100-ml Kjeldahl flask, the long neck of which is replaced by a 24-40 standard ground glass taper. The still head is the male portion of the taper, flared at the upper end to hold a No. 6, three-holed rubber stopper. A bent glass tube inserted through one hole admits compressed air, another holds the thermometer, and the third admits the end of the U-shaped distilling arm that leads to the receiving bottle.

For the usual sample of 500-750 mg of cacodylic acid, the distilling flask contains 7.5 cc of concentrated HCl (arsenic-free), and 0.4 g of cuprous chloride (CuCl). The cuprous chloride reduces any arsenic present in the pentavalent state to the trivalent form. Once the vial of cacodylic acid is added, the still head is sealed by manipulating the thermometer stem from above, through the roof of the shield. The flask is heated by a Glass-col* heating mantle and distillation is carried out to 110°C. Between 80-110°C the free trivalent arsenic is distilled as $AsCl_3$. The current of air, introduced through the still head, increases the yield. The distillate is caught in a graduated 30-ml bottle, containing 10 cc of cold, distilled water in a small ice-bath. The volatile $AsCl_3$ fumes, bubbling through the liquid, are hydrolyzed to arsenious acid. Neutralization to the phenolphthalein end point (pH 8.3) with 6 N NaOH follows. The NaOH is introduced through the long-stemmed burette that pierces the shield roof.

Aliquots for activity measurements and arsenic determinations are obtained with pipettes inserted in the remote control pipettor.[6] A 25-lambda micropipette withdraws a sample that is diluted in a 100-ml volumetric flask with 6 N arsenic-free nitric acid. Five cc of this solution is further diluted to the mark with nitric acid in another 100-cc volumetric flask. Four-cc samples of this final dilution are placed in duplicate Coors porcelain capsules and evaporated to dryness. These samples are then counted under a thin-window mica Geiger-Mueller tube and aluminum foil absorber of 54 mg/cm^2, using a conventional scaling and timer unit, and compared with a standard uranium source which is similarly mounted. Because of the high energy levels of the beta and gamma radiations of the arsenic sample (β max = 3.04, γ max = 2.15), mass absorption can be neglected.

The activity of the sample is calculated as follows:

$$\mu c\ As^{76}/ml = \frac{c/s\ sample}{c/s\ standard} \times \frac{1}{\text{Absorption factor Al foil} \times \text{dilution factor}} \Big/ \frac{}{3.7 \times 10^{-4} \times \text{geometry factor of standard}}$$

The total amount of arsenic present, both stable and radioactive, is determined by means of the modified Gutzeit method for arsenic, as described in Scott's Standard Methods of Chemical Analysis.[7] Usually 0.1 cc of the solution obtained is sufficient to give a 20-30 μg stain. Knowing this value and the activity of the sample, the "specific activity", *i.e.*, the ratio of the number of radioactive arsenic atoms to the total number of arsenic atoms, is obtained.

Results. Table I is a summary of several

* Obtainable from the Glass-col Apparatus Co., 1700 S. 7th Street, Terre Haute, Indiana.

[6] Tompkins, P. C., Broido, A., and Teresi, J. D., The Handling of Radioactive Materials in the Experimental Biology Section, Manhattan District Declassified Document (MDDC) 377.

[7] Scott, W. W., *Standard Methods of Chemical Analysis*, Van Nostrand Co., New York, 5th Edition, Vol. I, pp. 101-108, 1939.

TABLE I.
Yields of Radioactive Arsenic^{76} from Pile Irradiated Cacodylic Acid (Szilard-Chalmers Reaction).

Amt. cacodylic acid irradiated (mg)	NaCl %	Activity/cc (mc)	Arsenic/cc (mg)	Total vol. (cc)	Total activity (mc)	Specific activity (mc/mg)
300	10	2.37	0.09	17.5	41.5	26.4
700	14	4.26	0.26	28.0	119.0	15.2
500	13.0	4.35	0.35	24	104.4	12.0
600	7.7	4.50	0.35	20	90.0	12.8
700	10.0	8.50	0.34	17	142.0	25.0
750	16.0	5.60	0.32	20	112.0	17.5
800	20.0	4.57	0.26	16	73.2	17.5
500	16.0	2.77	0.17	17	47.1	16.4
800	14.0	4.0	0.26	25	100.0	16.9
650	14.0	6.48	0.20	25	162	32.4
700	11	6.82	0.18	8	54.6	30.3

TABLE II.
Comparison of Specific Activities of Several Arsenic Compounds Exposed to Pile Irradiation.

Material	mc As^{76} / mg As^{75}	Enrichment
As_2O_3	0.5	1
Cacodylic acid $(CH_3)_2AsO(OH)$	15.0	30
Arsenilic acid $C_6H_4NH_2AsO_3H$	0.1	0.2

Values are representative ones.

preparations of arsenic^{76} from pile irradiated cacodylic acid. There is wide variation in the specific activities because (1) time and power levels of pile irradiation were not uniform, and (2) activities were calculated to time of preparation of the arsenic solution.

It will be seen, however, that specific activities of arsenic^{76} obtained by pile irradiation of cacodylic acid are considerably higher than those obtained by pile irradiation of arsenic trioxide. Table II compares specific activities following irradiation of cacodylic acid, arsenic trioxide, and arsenilic acid. In addition to the high specific activity of the resulting product, as can be seen from Table I, irradiation of cacodylic acid yields a solution of high total activity, this being a function of the high neutron capture cross section for As^{75}.

Summary. A method of obtaining As^{76} of high specific activity by pile irradiation of cacodylic acid is described.

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Relation of Folic Acid and Vitamin A to Incidence of Hydrocephalus in Infant Rats.*

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Richardson and Hogan[1] observed hydrocephalus among infant rats and ascribed it to the inadequacy of the maternal diet. The diet, which was of the usual synthetic type, was supplemented with all of the recognized

* Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Station, Journal Series No. 1130.

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[1] Richardson, L. R., and Hogan, A. G., *J. Nutrition*, 1946, **32**, 459.