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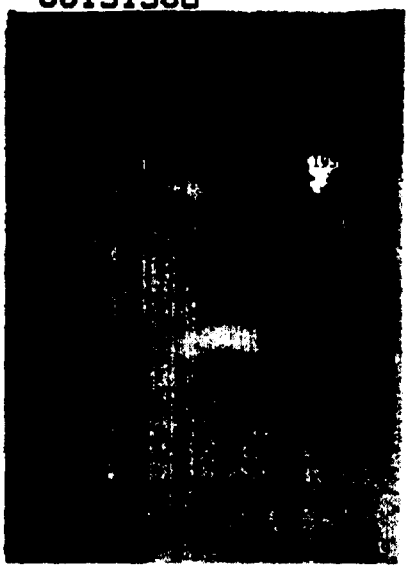

Fig. 1. Density gradient tubes with ion exchange resins in sodium tungstate solutions. (Left) Dowex 50-W. (Right) Laboratory preparations of sulfonated polystyrene resins.

and the homogeneity of the resins used is obviously very important. We have used the density gradient method of Linderström-Lang (*2*) to study this homogeneity. A tube was set up which had two bulbs, each of about 100-ml capacity, joined by a vertical tube 2.5 cm in diameter and 18 cm long. A solution of sodium tungstate which was dense enough to float the resins was poured into the lower bulb and the lower half of the vertical tube. Over this, filling the upper bulb, was poured a more dilute solution of sodium tungstate which was not dense enough to float the resins. The tube was set in a thermostat at 25° ± 0.01°C and left for 2 days. The solutions mixed by diffusion, and a stable gradient of concentration and density was established along the length of the vertical tube.

Small portions (about 50 mg) of the resins to be studied were then dropped into the tube. These were sulfonated polystyrene resins converted to the sodium form and air-dried. Within an hour they came to rest within the vertical tube, the more highly cross-linked resins settling further down the tube, and they remained in the same position for days.

Figure 1 is a photograph of two such tubes. On the left are two commercial resins supplied by the Dow Chemical Co. (Dowex 50-W, 50–100 mesh), with 4 and 8 percent cross linking, respectively; the 4-percent cross-linked resin is on top. As may be seen, these resins are remarkably uniform, though both contain a small amount of light material which floats just above the main quantity of resin. On the right are three laboratory-scale batches with nominal 7, 10, and 17 percent cross linking, respectively. These are less homogeneous.

To determine the density distributions in the vertical tubes, small portions of solution (0.2 to 0.5 ml) were withdrawn from measured levels by a pipet with a long capillary tip, and their densities were measured by a micropyknometer. Or, their refractive indices were measured and compared with those of solutions of known concentration and density. Typical data are given in Table 1.

Sodium tungstate (Na_2WO_4) was chosen as the heavy solute because it is the anion which is dense, and anions are partially excluded from a cation exchange resin. Another heavy solute which worked well was disodium lead ethylenediamine tetraacetate (Na_2PbY).

The density at which the resin floats is determined by its swelling, which in turn depends on two factors, cross linking and extent of sulfonation. The "laboratory batch" resins were somewhat lighter than the commercial resins of comparable cross linking, a finding which suggests that they were more highly sulfonated. That this was, in fact, the case is seen from the ion-exchange capacities reported in Table 1. A resin could appear homogeneous by the flotation test yet not be truly homogeneous; by using more than one flotation solute it may be possible to distinguish between the effects of cross linking and sulfonation.

The flotation technique has obvious potentialities for producing very uniform batches of resin (*3*).

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Table 1. Concentrations, densities, and refractive indices of solutions floating sulfonated polystyrene ion-exchange resins.

Resin		Na_2WO_4			Na_2PbY		
Cross-linking (%)	Exchange capacity*	Sp. gr. (25°C)	n^{25}_D	WO_3 (wt.%)	Sp. gr. (25°C)	n^{25}_D	Pb (wt.%)
			Dowex 50-W				
4	4.78	1.302	1.3688	28.0	1.298	1.3960	13.35
8	4.48	1.338	1.3718	30.65	1.333	1.4016	14.92
			Laboratory batches				
7	4.60	1.322	1.3701	28.2			
10	4.425	1.347	1.3728	31.55			
17		1.383	1.3768	35.0			

* Exchange capacities are in milliequivalents per gram of dry sodium resin.

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3. This work was supported by the U.S. Atomic Energy Commission [contract AT (11-1)-499]. The laboratory batches of resin were provided by C. Calmon of the Permutit Company, whom we wish to thank.

29 October 1959

Zinc-65 in Cyclotron Workers

Abstract. Small but persistent body burdens of zinc-65 have been found in cyclotron workers. This radionuclide and others are produced by nuclear reactions with the construction materials of the cyclotron. Only zinc-65 has gained entry into the body, but in amounts of less than 1 percent of the maximum permissible amount.

Measurement of Los Alamos Scientific Laboratory cyclotron workers in November 1958 in the human counter (*1*), a large liquid scintillator designed for detection of radioactivity in human beings, revealed the presence of radioactivity in excess of normal potassium-40 activity in the 1- to 2-Mev region. This excess was identified as zinc-65 in the Los Alamos human spectrometer (*2*), a large NaI (Tl) crystal in a steel room by means of which radioactivity in human beings can be characterized. Figure 1 is a typical spectrum showing the characteristic zinc-65 peak at 1.11 Mev in addition to the usual cesium-137 and potassium-40 peaks.

Zinc-65 can be produced in large quantities in cyclotrons accelerating deuterons by the reaction Cu^{65} (*d*, 2*n*)-Zn^{65} on copper dees and other parts. In fact, this reaction has been used for producing zinc-65 of high specific activity (*3*) for biological tracer work. In the acceleration of helium-3 ions, lesser amounts result from the reaction $Cu^{65}(He^3,H^3)Zn^{65}$, and in addition the reaction $C^{12}(He^3,2He^4)Be^7$ may occur if carbon (as graphite, oil, grease, and so on) is bombarded. Deuteron reactions on iron, chromium, and manganese can produce manganese-54, which also results from the *p,n* reaction on chromium-54. The properties of these radionuclides are summarized in Table 1. The Los Alamos variable-energy cyclotron is used to produce beams of protons (3.9 to 9 Mev), deuterons

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(6.2 to 15 Mev), helium-3 ions (9.3 to 24 Mev), and helium-4 ions (13.4 to 32 Mev). The copper dees are approximately 20 in. in radius and have ⅛-in. graphite liners to reduce induced radioactivity. Other parts [deflector, exit strips (septum), ion source cone, and feeler] exposed to beam bombardment contain chromium-plated copper and graphite. Hence, manganese-54 and beryllium-7 are possible products, in addition to zinc-65.

A repair operation in which the machine is opened and the dees are removed is referred to as a "rollback" at this laboratory. When this occurs, a major clean-up usually follows. Many parts are found to have carbon deposits and other foreign material. Cleaning these parts by use of solvents, grinding, or sanding contaminates the air, as shown by air samplers (*4*). Indeed, it appears that just opening the machine causes air contamination.

During rollbacks, gamma-ray spectra of filter papers from the air samplers have shown zinc-65 and, in addition, peaks very close to the energy of beryllium-7 (0.48 Mev) and manganese-54 (0.84 Mev). The surface of a nearby workbench top showed the same radioactivities, as did the cyclotron deflector and ion source holder. If the 0.48 Mev peak was actually beryllium-7, then beryllium-7 and zinc-65 were the predominant radionuclides observed, and their abundances were comparable. Tools (a screwdriver and so on) were [illegible]ly contaminated; the amount of [illegible]5 was of the order of 0.1 μc.

Gamma-ray spectra of several of the cyclotron personnel most involved in rollback operations have been measured serially since the exposure was discovered in November 1958. These data are shown in Fig. 2. The maximum body burdens observed were about 0.1 μc, a burden which is quite trivial compared with the maximum permissible body burden of 60 μc (*5*). Recontamination has undoubtedly occurred in the interval studied, due to rollbacks and general area contamination, as the data show. However, the results are consistent with the information on zinc-65 metabolism recently obtained at this laboratory (*6*). Doses administered orally were efficiently absorbed, and retention ranged from 50 to 80 percent several months after ingestion; urinary excretion several weeks after ingestion was only about 0.05 to 0.1 percent of the body burden. Analysis of urine samples of the five cyclotron workers listed in Fig. 2 showed that if any zinc-65 were present, the amount was less than 0.2 to 0.3 percent of the body burden in a 24-hour sample. It is interesting to note that only once did the cyclotron personnel show any radioactivity other than zinc-65 and the usual cesium-137 and potassium-40 activity. This was at the end of June 1959, when they showed a peak at 0.46 Mev, which is very close to the beryllium-7 energy. This may have been from skin or hair contaminants subsequently washed off.

Table 1. Properties of some cyclotron-produced radionuclides.

Isotope	Mode of decay	$T_{1/2}$ (days)	E_γ (Mev)	N_γ/N_d	Air MPC* for 40-hr wk ($\mu c/cm^3$)
Be^7	E.C.	53	0.48	0.12	10^{-4}
Mn^{54}	E.C.	291	0.84	1.00	8×10^{-7}
Zn^{65}	E.C. (98%)				
	$\beta+$ (2%)	245	1.11	0.51	10^{-7}

*Maximum permissible concentration.

To minimize recontamination during rollbacks when particulate matter may become airborne, protective shoe coverings, gloves, coveralls, caps, and respirators are used. The routes of entry into the body have not been determined with certainty, but probably one means of contamination is inhalation. All the personnel were fitted for respirators (the half-mask type being preferred by the group). External exposure has been kept within permissible levels through shielding, distance, and limitation of working time.

It is probable that many cyclotron workers and perhaps individuals working on other accelerators (or reactors) have small burdens of zinc-65. Such burdens have been reported in two cyclotron workers at Massachusetts Institute of Technology (*7*). It would be of interest to survey those potentially exposed at other installations, from the

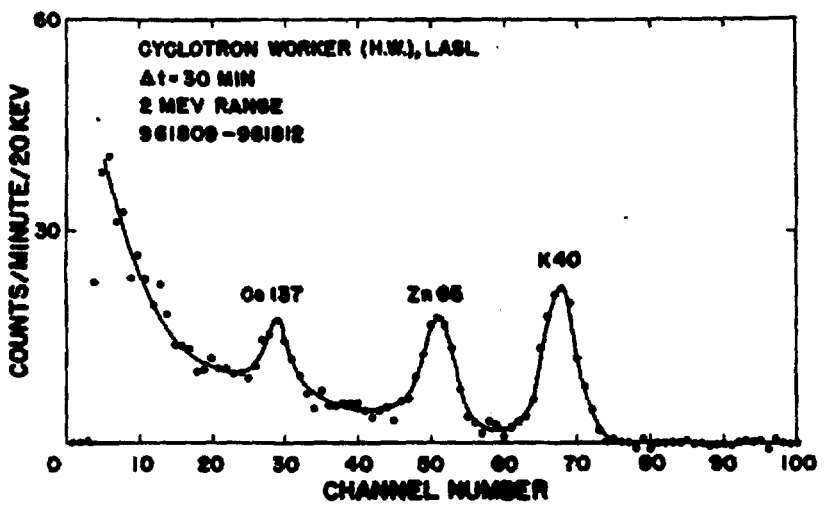

Fig. 1. Gamma-ray spectrum of cyclotron worker (H.W.) with zinc-65 burden.

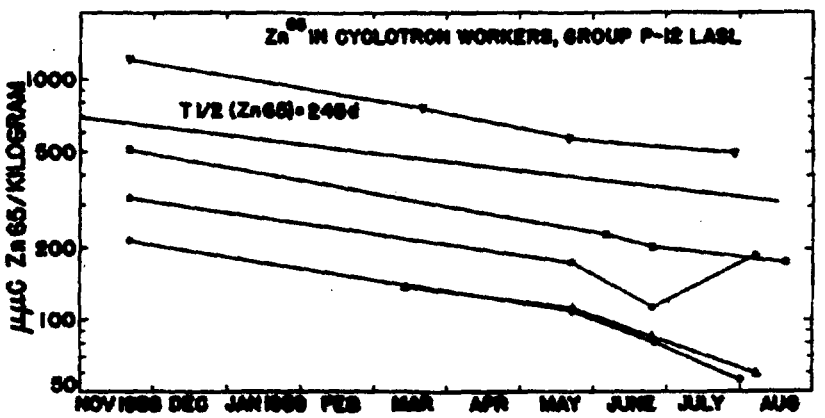

Fig. 2. Body content of zinc-65 as a function of time in five cyclotron workers (▼, D.A.; ■, H.W.; ●, R.C.; ○, D.C.; and ▲, J.N.).

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standpoint of good health-physics practice and for better understanding of the metabolism of radionuclides in the human body. To make such surveys is becoming increasingly easier, since human spectrometers and whole-body counters have been or are being built at many universities and atomic energy installations throughout the world (*8*).

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2 November 1959

Laminarase of Euglena gracilis

Abstract. An enzyme in extracts of the protistan *Euglena gracilis* splits the polysaccharide laminarin (β-1:3-glucosan). Its optimal *p*H is 5.0, and it is activated by Mn^{++} ions.

The storage polysaccharide (paramylon) of the protistan *Euglena gracilis* has been reported to be a β-1:3-glucose polymer (*1*) probably very similar in structure to laminarin (*2*), a polysaccharide produced in large quantities by several species of Phaeophycea (brown algae), notably *Laminaria* (*3*). Because of this similarity it seemed of interest to look for a laminarin-splitting enzyme in *Euglena*. Laminarase has been described in extracts of higher plant tissues (*4, 5*).

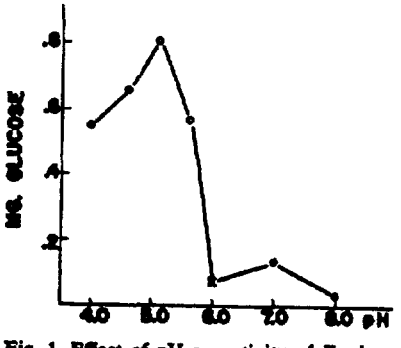

Fig. 1. Effect of *p*H on activity of *Euglena* laminarase. Assay was carried out as described in the text, but without Mn^{++}. Final buffer concentration, 0.1*M*. Open circle, acetate; cross, phosphate; solid circle, "Tris" (*7*).

Euglena gracilis (strain Z, Indiana collection strain No. 753) was grown as described elsewhere (*6*). The cells were harvested by centrifugation, suspended in approximately 5 vol of acetone at −15°C, homogenized for 30 seconds in a Waring blender, collected by filtration and washed with additional cold acetone, and given a final ether wash until most of the chlorophyll had been removed. The acetone powder was further dried overnight under reduced pressure. Extraction of the enzyme was carried out by stirring 5 gm of the *Euglena* acetone powder for 4 hours at 4°C in 50 ml of 0.04*M* acetate buffer, *p*H 6.0, containing NaCl at a concentration of 0.5*M* and Versene at 0.002*M*. The extract was dialyzed overnight against 0.01*M* "Tris" buffer (*7*), *p*H 7.3. The laminarase assay was based on the colorimetric determination of the reducing groups liberated by the action of the enzyme on the polysaccharide with 3,5-dinitrosalicylic acid, a reagent much used for the determination of amylase (*8*). The final conditions of the laminarase assay were as follows: 5 mg of laminarin (*9*) was incubated with occasional stirring for 2 hours at 37°C with 0.25 ml of the *Euglena* extract, in a final volume of 1.0 ml of 0.1*M* acetate buffer, *p*H 5.1, containing $10^{-3}M$ Mn^{++}. The reaction was stopped by the addition of 1.0 ml of the dinitrosalicylic acid reagent (*8*) and 3 ml of water. The excess laminarin was removed by centrifugation, and the supernatant was heated in a boiling-water bath for 5 minutes. After cooling, the volume was adjusted to 10 ml, and the color intensity was determined with a Klett-Summerson photoelectric colorimeter with a green filter (No. 54). The amount of reducing material liberated was expressed as milligrams of glucose.

The optimal *p*H of the *Euglena* laminarase was found to be 5.0, as shown in Fig. 1. It is of interest that the same value has been reported for the enzyme from higher plant tissues (*5*). Another similarity was the relative heat lability of the *Euglena* enzyme: at 55°C and *p*H 7.3, 61 percent of the activity was lost in 4 minutes, while complete inactivation occurred within 8 minutes. A time course of the laminarase reaction is given in Fig. 2. When the effects of various metal ions on the enzyme were tested Fe^{+++}, Cr^{+++}, Ni^{++}, Ba^{++}, and Mg^{++} were

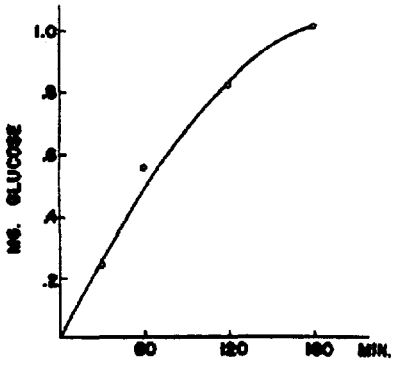

Fig. 2. Time course of laminarase reaction. Assay was carried out as described in the text, but without Mn^{++}.

found to be slightly inhibitory or inactive, while Hg^{++} was strongly inhibitory at concentrations of 10^{-4} and $10^{-5}M$. It was of particular interest that 10^{-4} and $10^{-5}M$ Mn^{++} strongly stimulated the enzyme. Activations from 50 percent to over 100 percent, depending on the enzyme preparations, were observed. Smaller activations resulted from the addition of Co^{++} or Ca^{++} at the same concentrations.

Fractionation experiments now in progress tend to indicate that more than one enzyme may be involved in the breakdown of laminarin. Examination of the products of the enzymatic degradation may further clarify this point. In *Euglena*, it appears that laminarase probably serves to mobilize the reserve carbohydrates, especially since we were unable to find any amylase activity in the organisms (*10*).

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9. We wish to thank Stanley Peat, University College of North Wales, Bangor, and E. T. Dewar, Institute of Seaweed Research, Inveresk, Midlothian, for their generous gifts of laminarin.
10. We wish to thank M. K. Bach of this department for advice and help in growing the *Euglena*. The competent assistance of Mary B. Wall is gratefully acknowledged.

4 November 1959

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