and the tissue. Since the glass coating is thinnest at the tip, where an applied voltage has its strongest gradient, preferential breakdown of the insulation at this location is easily done electrically. The best electrical stability and lowest impedance may be obtained by coating the exposed area with platinum black. Fortunately, the two procedures may be combined into one by utilizing the platinizing voltage as the insulation rupturing voltage. The platinizing bath is a 1 percent solution of platinous chloride (stronger solutions appear to be detrimental to the coating). Current from a 15-volt d-c source in series with a 1-megohm resistor is passed for 15 to 30 seconds between the electrode and a platinum wire in the solution, the electrode being negative. A stream of tiny bubbles from the tip indicates a good electrode. Bubbles elsewhere indicate that the insulation is leaky and that the electrode needs to be recoated with glass. After electrodes have been used in biological material, they should be cleaned by bathing them overnight in distilled water.

Electrodes of this type have recorded successfully from the ganglion cells of the vertebrate retina, the optic nerve of the squid, mechanoreceptors on the antennae of mosquitoes, and many others. Some of the neurons recorded from were sensory nerve fibers and ganglion cells less than 10 μ in diameter for which most other types of electrodes are too noisy. Neurons giving impulses of 40 μv or more could easily be isolated from the surrounding neural activity. Figure 1 shows records made by using the same electrode first in the retina of a goldfish and then in the retina of a frog. With our recording system the electrode usually has a noise level of 20 μv and will discriminate impulses larger than 40 μv.

The electrode is durable enough to record well after penetrating the cartilaginous "skull" of a squid (*8*), and it should also be able to record successfully after penetrating other tough structures such as the dura of the mammalian brain (*9*).

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Isolation of Uridine Diphosphate–Glycosyl Compounds from the Slug

Abstract. An examination of the acid-soluble nucleotides of *Limax maximus* Linné revealed, among others, several uridine diphosphate–glycosyl compounds. Nucleotides isolated and identified were the uridine diphosphates of glucose, acetylglucosamine, and acetylgalactosamine.

Numerous reports have indicated the occurrence in various snails and other mollusks of sulfated polymers (mucins) which contain one or more of the sugars glucose, galactose, glucosamine, and galactosamine (*1*). Since current research trends support the hypothesis that polymer syntheses are intermediated by nucleotide-activated precursors (*2*), it seemed worth while to investigate the nucleotide content of the slug, *Limax maximus* Linné (*3*).

Nucleotides were extracted from 24 slugs (156 g) by homogenization with 150 ml of ice-cold 10-percent trichloroacetic acid in a Waring blender. An equal volume of ethanol was added, the precipitate was centrifuged off and discarded, and the supernatant solution was treated essentially by procedures previously described (*4*). Nucleotides in the extract were freed of salts by charcoal treatment (*5*) and then adsorbed onto a Dowex-1-formate column. The column was washed with 3*M* formic acid and the uridine diphosphate–glycosyl compounds were then eluted with 4*M* ammonium formate (*p*H 2.8).

Salts were again removed by charcoal treatment and the nucleotides were chromatographed on Dowex-1-formate with a linear gradient (*6*) which changed from 3*M* formic acid to 4*M* ammonium formate, *p*H 3. A single peak of the five obtained from the column contained over 80 percent (42 μmole as uridine) of the 260-mμ absorbing material. Only the material from the single large peak was further investigated. After processing by the charcoal procedure (about 25 percent recovery), the material gave 250 mμ to 260 mμ and 280 mμ to 260 mμ ratios of 0.76 and 0.42, respectively, thus suggesting the presence of uridine compounds. This material was then separated into three major ultraviolet absorbing bands by paper chromatography on Whatman No. 1 paper, with ethanol-M ammonium acetate (7.5:3, vol/vol) as solvent, first at *p*H 7.5 and then at *p*H 3.7 (*7*).

The slowest moving band contained at least 30 percent uridine diphosphate–glucose, as determined by comparing 260 mμ measurements with assay by uridine diphosphate–glucose dehydrogenase (*8*) and acid hydrolyzable glucose content as determined with glucose oxidase (*9*). The remainder of the material in the first band has not been identified.

The second band exhibited R_F values on paper chromatograms similar to known uridine diphosphate–acetylglucosamine and was found to contain acid-hydrolyzable acetylhexosamine (0.1*N* HCl/10 min at 100°C) (*10*). To determine whether or not more than one amino sugar was present, the material was hydrolyzed for 2 hours in 2*N* HCl and then was chromatographed on a 1- × 50-cm Dowex 50-H column by the procedure of Gardell (*11*). Two hexosamine-positive peaks (*12*) emerged from the column with R_F values suggestive of glucosamine and galactosamine, and were further identified as such by (i) comparison of R_F values with knowns on paper chromatograms with use of *n*-butanol-pyridine water (6:4:3 vol/vol) as solvent and silver nitrate to visualize sugar spots (*13*), and (ii) by degradation with ninhydrin by the procedure of Stoffyn and Jeanloz (*14*) to yield arabinose and lyxose, respectively.

The third, fastest-moving ultraviolet absorbing band contained a reducing compound (*15*) which has not been identified.

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A Difference between Biological Effects of Gamma Rays and Heavy Ions

Abstract. When irradiated with gamma rays, *Artemia* eggs show the typical sigmoidal survival curve of a multicellular organism, with little change at low doses and an abrupt decrease in survival above a threshold dose. On irradiation with 160-Mev oxygen ions, the threshold disappears and viability can be destroyed by passage of a single energetic ion.

Gamma-ray survival curves for many multicellular organisms are sigmoidal and show an initial insensitivity to low doses. Only above a threshold does the viability drop appreciably, and then it usually falls off quite rapidly. The ability of eggs of the common brine shrimp, *Artemia salina*, to hatch after gamma-ray exposure decreases in this way with increasing dose, as shown in Fig. 1. These eggs are moderately complex. The fertilized oöcyte divides to the blastula stage before becoming encysted and laid. In this stage the egg is about 200 μ in diameter, and it must dry before further development can take place. In all the experiments reported here the eggs were irradiated in high vacuum. There was no adverse effect from vacuum treatment alone. On immersion in sea water the egg develops rapidly, and at about 48 hours the shell cracks open and an embryo encased in a membrane is released. This process, called "emergence," is inhibited by radiation, as shown in Fig. 2. After another 6 to 8 hours a free-swimming larva comes out of the membrane; this step is called "hatching."

The long plateau which indicates the accumulation of gamma-ray damage is markedly reduced if the eggs are irradiated with 40-Mev helium ions and disappears entirely when 160-Mev oxygen ions are used. The particles were from the Yale heavy-ion linear accelerator, and dosimetry was carried out by methods previously described (*1*). The exponential decrease in survival indicates that the passage of a single energetic oxygen ion is responsible for the loss of activity of an egg. The result is qualitatively similar to that of Stapleton, Hollaender, and Martin with *Aspergillus* spores (*2*) but is more spectacular.

The three types of radiation differ from each other in the spacing between inactivating events, ranging from several thousand angstroms for gamma rays, to the order of tens of angstroms for 40-Mev helium ions (240 ev of energy loss per 100 angstroms of track), to angstroms for 160-Mev oxygen ions (3800 ev per 100 angstroms, or an average of 1.3 ion pairs, at 30 ev per ion pair, per angstrom of path). The effect is not caused by over-all dose rate, since (i) fast electrons given at a dose rate of 2 Mrad/min produced the same survival curve as gamma rays at 0.275 Mrad/hr, and (ii) the threshold was lower with helium ions and disappeared with oxygen ions, although the time required to deliver the total dose in the last two cases was about the same (tens of seconds).

Four possible explanations for the loss of a threshold with heavy ions are as follows.

1) The gamma-ray curve may be interpreted as showing that either a certain number (20 to 60) or a certain fraction of functioning units in the egg must be damaged to prevent development. It might be assumed that a single fast oxygen ion might do the necessary damage. However, converting the doses in Figs. 1 and 2 to particles per unit area shows that 24 and 9 oxygen ions per square micron are needed to suppress emergence and hatching, respectively, to 37 percent of that of controls. Each cell will have been traversed on the average by many ions before inactivation. Thus, the chance that any single ion will do all the necessary damage is small, and on this assumption the survival curves with oxygen ions would still show a cumulative effect, not the exponential form found.

2) The *simultaneous* inactivation of several widely separated areas by a heavy ion might be biologically more effective than consecutive inactivation by gamma rays. This is a process which might be very important in a metabolizing system, especially at low dose rates, but it is doubtful that it was important in dried eggs.

3) If the inactivating events took place close enough together in the densely ionizing track of an oxygen ion the resultant physicochemical events might be different, and conceivably more effective. There is some evidence for this process from experiments with heavy ions on dried enzymes (*3*), and it could be operative here.

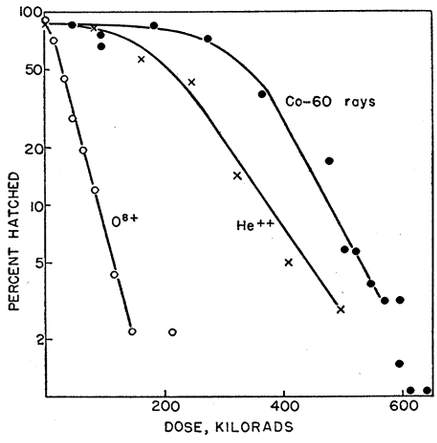

Fig. 1. Plot of the percentage of *Artemia* eggs hatched (semi-log scale) against dose for three different radiations.

4) The most likely possibility is that if enough damage is done within some limited volume the egg will not develop. This damage can be cumulated through many "hits" from gamma rays or caused by a single oxygen ion. Presumably the dimensions of this volume are less than 1 μ. There may be several such volumes per egg.

An extrapolation of this result leads one to consider the possibility that heavy ions in the primary cosmic rays which are met with above the earth's atmosphere may produce radiological effects at low total dose levels which would not be expected from x-ray data because of threshold effects. This suggestion is contrary to the conclusion advanced by Zeman, Curtis, Gebhard, and Haymaker (*4*) from their work with microbeams of deuterons on mouse-brain tissue. *Artemia* is an unusual material, as shown by its extreme

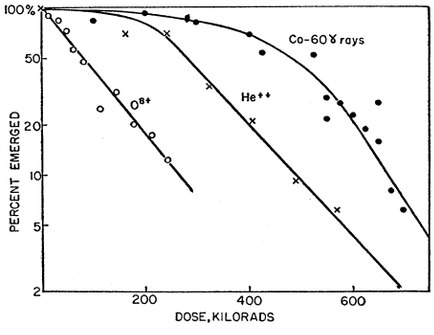

Fig. 2. Plot of ability of the *Artemia* larva to emerge from the egg (the first stage in development) against dose for three different radiations.