An additional safety factor, in the form of a one inch glass tube, is installed to accommodate any backflow of water between the mercury trap and filter pump.

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THE PREPARATION OF PERMANENT SLIDES OF THE RHIZOPOD ARCELLA

HEGNER[1] has recently described a convenient method for fixing and preserving specimens of the shelled rhizopod Arcella. By his method, Schaudinn's alcoholic-acetic-sublimate is poured over the animals while they are attached to the bottoms of Petri dishes, and clearing and preserving are effected by the use of glycerin.

We use a similar method in the preparation of slides of Arcella in our protozoology course, and it is believed that a note upon the method will be of interest, for it supplements Hegner's method in that it concerns the staining of the arcellas as well as their fixation. The procedure follows.

The arcellas, together with several drops of fluid, are transferred from a hay-infusion stock culture to clean micro slides. The slides are set aside in a moist chamber and are left undisturbed for at least half an hour. In this interval, many of the arcellas usually settle to the bottom of the fluid and attach to the slides by means of their pseudopodia.

The surplus fluid on a slide is now poured off. This operation leaves the animals still attached to the slide and covered only by a relatively thin film of fluid. Schaudinn's solution (used cold) is now dropped directly on the arcellas by means of a pipette and is left on the slide for two or three minutes. This method of fixation leaves the animals attached to the slide, frequently with pseudopodia extended. Thus the common difficulty of cementing protozoa to the slide is circumvented by the behavior of the arcellas themselves.

The slide is now ready for staining, and in this process it is treated entirely like a histological preparation. It is immersed in succession in the following fluids: 70 per cent. alcohol, made light brown by the addition of tincture of iodin (to remove sublimate), 30 minutes; 50, 25 per cent. alcohol, water, 1 to 3 minutes in each; dilute Delafield's hematoxylin (1 part stock Delafield to 3 parts water), 5 to 10 minutes; water, 25, 50, 70, 95 per cent. alcohol, absolute alcohol, equal parts absolute alcohol and xylol, pure xylol, 1 to 3 minutes in each; balsam.

If desired, Heidenhain's iron hematoxylin may be used instead of Delafield's. In this case, after fixation and treatment with iodin, the slides are hydrated, and are then mordanted an hour in 4 per cent. ammonio-ferric sulphate (iron alum). They are stained at least four hours in 0.5 per cent. aqueous hematoxylin solution and are destained in 2 per cent. iron alum, after which they are dehydrated, cleared and mounted.

In preparations stained by either method, the nuclei, of which there are two in most species, each with a conspicuous central karyosome, are revealed with diagrammatic clearness. The shell aperture and the extended pseudopodia are likewise well shown. Arcella is particularly favorable for the demonstration of chromidia, and by this method the chromidial bodies—commonly overlooked by students—are rendered clearly visible.

In conclusion, the method affords a convenient procedure for demonstrating certain cell organelles which are not readily observed in the living animal. Furthermore, the permanency of such preparations makes them available for class study or demonstration at times when living arcellas are not immediately obtainable.

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SPECIAL ARTICLES

ACTINO-URANIUM AND THE RATIO OF ACTINIUM TO URANIUM IN MINERALS

VARIOUS speculations have been advanced in the past about the origin of actinium.[1] However, the experimental evidence in all cases is fundamental. This evidence is (*a*) Boltwood's original work[2] on the "constancy" of actinium to uranium in several uranium-bearing minerals from North Carolina, all presumably of the same age; (*b*) the very important recent work of Aston on the relative intensities of the mass spectral lines Pb_{208}, Pb_{207}, Pb_{206} from the lead isotopes of a bröggerite from Karlhus, Raade, Norway,[3] and also Aston's similar work on the ordinary lead,[4] and (*c*) the observations of various investigators that the relation of actinium to uranium seems to vary somewhat in minerals which may be of different ages. Among these the most recent work is that of Wildish.[5]

Rutherford[6] using Aston's data and making certain

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[1] Robert Hegner, *Trans. Am. Micr. Soc.*, 48: 214, 1929.

[1] Résumé in *Radioactivity Bull. N.R.C.*, No. 51, 1929.

[2] B. B. Boltwood, *Am. J. Sci.* [4] 25: 269–298, 1908.

[3] F. W. Aston, *Nature*, 123: 313, 1929.

[4] F. W. Aston, *Nature*, 120: 224, 1927.

[5] James E. Wildish, *J. Am. Chem. Soc.*, 52: 163–177, 1930.

[6] Sir Ernest Rutherford, *Nature*, 123: 313, 1929.

assumptions deduced the half-value period of actino-uranium as $T = 4.2 \times 10^8$ years.

Piggot[7] expressed the opinion that if the relative intensities of the lead isotope lines 208, 207, 206 are known the age of the mineral can be accurately determined. He was instrumental in having the bröggerite lead and also the ordinary lead material prepared for Aston's analysis. On the basis of Aston's results on the bröggerite lead and their own chemical analysis of the mineral from which the lead was obtained, Fenner-Piggot[8] calculated the age of the mineral considering the 208, 207, 206 intensities as indicative of the relative amounts of Th D, Ac D and Ra G. They found, however, that the ages calculated on the basis of the uranium content and on the basis of the thorium content were in great disagreement. When one considers the probable error given by Aston for the intensities and also the small amount of thorium, one would expect some disagreement. However, any common lead present will introduce a very serious error in the calculations on the basis of the thorium content because the Pb_{208} line in the ordinary lead is one half of the total intensity, and if it is considered (in the calculations) as of Th D origin the age must come out greater than it should, and since the Th amount is relatively small, the error is proportionately greater.

In the work of the committee on the age of the earth of the National Research Council, I have frequently pointed out the error of neglecting the possible presence of common lead. I have developed formulas for the calculation of this amount and the age of the mineral basing the equations on the two fundamental facts, namely: I. The sum of the atoms of common lead (L), Ac D, Ra G, Th D equals the number of atoms in the lead isotopes found in the mineral; II. The sum of the masses of the common lead (L), Ac D, Ra G, Th D is equal to the total mass of the lead isotopes; and, of course, using the radioactivity equations of disintegration and of equilibrium. If the actinium series is considered as a branch from U II the equations obtained are

$$\frac{1.20\,U}{238.17}\cdot\left(e^{\lambda_u\cdot t}-1\right)-\frac{0.80\,Th}{232.15}\cdot\left(e^{\lambda_{th}\cdot t}-1\right) = Pb\cdot\left(\frac{207.20-A}{A}\right)$$

and

$$L = 207.20\left[\frac{Pb}{A}-\frac{U}{238.17}\cdot\left(e^{\lambda_u\cdot t}-1\right)-\frac{Th}{232.15}\cdot\left(e^{\lambda_{th}\cdot t}-1\right)\right]$$

in which U, Th, Pb and L are masses in grams of uranium, thorium and lead isotopes, and ordinary lead, respectively; and for the atomic weights: A refers to the lead isotopes and 206, 208 are used for Ra G (also Ac D) and Th D respectively. These equations presented at the Washington meeting of the American Physical Society in 1929[9] will be published in the report of the National Research Council committee and also in the *American Journal of Science.*

In this case L represents accurately the amount of common lead. If, however, the actinium descends from actino-uranium, an independent isotope of uranium, some Ac D is included in the value L and also a part of Ac D is included with Ra G. The part with Ra G depends on the actinium: uranium ratio, r. Then, if $u \equiv$ number of atoms of uranium, $r \cdot u$ in final disintegration would be actinium lead atoms. The amount of Ac D thus considered with Ra G is

$$\frac{r\cdot U}{238.17}\cdot\left(e^{\lambda_u\cdot t}-1\right)\cdot 206.$$

This correction can be readily made when we are certain of this lineage and also of the value of the ratio, r.

Using Fenner-Piggot's analysis data, namely, $U = 61.158$, $Th = 4.377$ and $Pb = 8.018$, and Aston's $A = 206.19$, and assuming $r = 0.03$ and making the above corrections for Ac D we obtain for the age of this bröggerite $t = 825 \times 10^6$ years and for $L' = L + Ac\,D = 0.986$ gm per 100 gm mineral. Now,

$$Ra\,G = \frac{0.97\,U}{238.17}\cdot\left(e^{\lambda_u\cdot t}-1\right)\cdot 206 = 6.856$$

$$Th\,D = \frac{Th}{232.15}\cdot\left(e^{\lambda_{th}\cdot t}-1\right)\cdot 208 = 0.176$$

Using Aston's relative intensities $Pb_{206} : Pb_{207} : Pb_{208} = 100 : 10.7 \pm 3 : 4.5 \pm 2$ we get $Pb_{206} = 6.960$, $Pb_{207} = 0.745$ and $Pb_{208} = 0.313$ gram. For the ordinary lead Aston gives the intensities $I_{206} : I_{207} : I_{208} = 4 : 3 : 7$. Hence we can write

$$Pb_{208} = 0.313 = Th\,D + \frac{1}{2}L \text{ (ord. lead)} = 0.176 + \frac{1}{2}L \quad \ldots\ldots (1)$$

$$Pb_{207} = 0.745 = Ac\,D + \frac{3}{14}L \quad \ldots\ldots (2)$$

$$Pb_{206} = 6.960 = Ra\,G + \frac{2}{7}L = 6.856 + \frac{2}{7}L \quad \ldots\ldots (3)$$

and

$$Ac\,D + L = 0.986 \quad \ldots\ldots (4)$$

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[7] C. S. Piggot, *J. Wash. Acad. Sci.*, 18: 269–273, 1928.

[8] C. N. Fenner and C. Piggot, *Nature*, 123: 793–794, 1929.

[9] A. F. Kovarik, *Phys. Rev.* [2] 33: 1069–1070, 1929.

The amount of common lead can be calculated separately from (1), (3) and (2) + (4) and comes out

$L = 0.274$ from (1)
$L = 0.364$ " (3)
$L = 0.307$ " (2) and (4).

In view of the probable errors in the intensities, these results for the amount of common lead must be considered quite satisfactory.

It is evident, therefore, that in calculating either the age of the mineral or the disintegration constant of actino-uranium one must take into consideration the ordinary lead present in the mineral. That common lead may be present in bröggerites has also been shown by Professor Ellen Gleditsch.[10]

The ratio, r, of actinium: uranium is really defined by the equation

$$r \cdot \lambda_u \cdot u = \lambda_{ac} \cdot ac = \lambda_{ac\,u} \cdot ac\,u_t = \lambda_{ac\,u} \cdot ac\,u_o \cdot e^{-\lambda_{ac\,u} \cdot t} \quad \ldots. \text{(I)}$$

in which u and ac refer, respectively, to the number of uranium atoms and the number of actinium atoms under conditions of radioactive equilibrium. In experimental work in which r was determined, the whole of uranium was taken as U, and hence

$$u = \frac{U}{238.17} \cdot N \quad \ldots. \text{(II)}$$

in which U is the mass of all the uranium and N is Avogadro's number.

If $ac\,u_o$ and $ac\,u_t$ represent, respectively, the number of atoms of actino-uranium present in the mineral initially and at the time, t (time of analysis), and since Ac D is the final product of actino-uranium, then

$$\frac{Ac\,D}{207} \cdot N = ac\,u_o \cdot (1 - e^{-\lambda_{ac\,u} \cdot t}) \quad \ldots. \text{(III)}$$

From the equations (I), (II) and (III) we obtain

$$\frac{Ac\,D}{207} = (\lambda_u \cdot \frac{U}{238.17} \cdot r) \cdot \left(\frac{e^{\lambda_{ac\,u} \cdot t} - 1}{\lambda_{ac\,u}} \right) \quad \ldots. \text{(IV)}$$

Substituting into (IV) values given above and using $\lambda_u = 1.52 \times 10^{-10}\ \text{yr}^{-1}$ and $r = 0.03$ we get

$$\lambda_{ac\,u} = 2.5_4 \times 10^{-9}\ \text{yr}^{-1},$$

or

$$T_{ac\,u} = 2.7_3 \times 10^8 \text{ years}$$

If we may assume that the relative amount of actino-uranium to uranium (I + II) is initially the same in all uranium-bearing minerals (an assumption offering some difficulties) then this relative amount should change with the progress of time and r should be less in older minerals at the time of the analysis. The results below show that such a variation seems to exist.

Let t′ be the age of the mineral for which $r = 0.03$ and t be the age of the mineral for which $r = 0.08$, then

$$\frac{r_{t'}}{r_t} = \frac{e^{\lambda_u \cdot \Delta t}}{e^{\lambda_{ac\,u} \cdot \Delta t}} = \frac{0.03}{0.08} = \frac{e^{1.52 \times 10^{-10} \cdot \Delta t}}{e^{2.54 \times 10^{-9} \cdot \Delta t}}$$

from which $\Delta t = t' - t = 4 \times 10^8$ years.

Boltwood, who obtained $r = 0.08$, used the North Carolina uraninites (Spruce Pine), and Hahn-Meitner, whose $r = 0.03$, used "pitchblende." Ages of the minerals are not given. Kirsch, whose value of r is 0.04 and more nearly like that of Hahn-Meitner's value, used Morogoro pitchblende and also a bröggerite from Raade, Moss, Norway, whose age is, at least approximately, 8 or 9×10^8 years. The determination of the age of a North Carolina uraninite (Flat Rock Mine) yields a very much lower age. If the ages were accurately known, it is not improbable that the difference in age may be such as to account for the different value of the ratio obtained by Boltwood and by Hahn-Meitner or by Kirsch or by S. Meyer.

Two minerals, namely, uraninite from Keystone, South Dakota, and uraninite from Sinyaya Pala, Carelia, supply data which with some assumptions, reasonable for these two minerals, may be used to produce a check on these deductions. The ages of these minerals by the above "age formula" come out, respectively, 1465×10^6 yr and 1852×10^6 yr and the corresponding values of L (= ordinary lead + some Ac D) are 0.67 and 0.91. Considering the great ages of these minerals and also the relatively more rapid rate of disintegration of actino-uranium than of uranium, it is highly probable that a greater part of the "L" is Ac D in the case of these minerals. In any case the error will not be great to assume this. Correcting also Ac D by a fraction

$$\frac{r \cdot U}{238.17}(e^{\lambda \cdot t} - 1) \cdot 206$$

we have

$$Ac\,D = L + \frac{r \cdot U}{238.17} \cdot (e^{\lambda_u \cdot t} - 1) \cdot 206$$

$$= 207 \cdot (\lambda_u \cdot \frac{U}{238.17} \cdot r) \cdot \left(\frac{e^{\lambda_{ac\,u} \cdot t} - 1}{\lambda_{ac\,u}} \right) \ldots. \text{(A)}$$

which can be solved for r. We can also calculate r from the difference of age of the mineral under consideration and the age of a mineral for which $r = 0.03$. For the latter age we may use the approximate value 8×10^8 years. We get

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[10] Ellen Gleditsch, *Skrift. Norsk. Vidensk. Akad. Oslo*, No. 3, pp. 1–49, 1925.

$$r = 0.03 \cdot \left(\frac{e^{\lambda_u \cdot \Delta t}}{e^{\lambda_{ac\,u} \cdot \Delta t}} \right) \quad \ldots . \text{(B)}$$

in which Δt = age of mineral considered minus 8×10^8 years.

The essential data for these minerals are:

Keystone, S. D., uraninite:
$U = 66.90$, $L' = 0.67$, $t = 1465 \times 10^6$ yr.
Sinyaya Pala, Carelia:
$U = 61.41$, $L' = 0.91$, $t = 1852 \times 10^6$ yr.

The results of such calculations are:

Mineral	r from (A)	r from (B)
Uran. Keystone, S. D.	0.0053	0.0061
Uran. Sinyaya Pala	0.0027	0.0024

We see that the two calculated values in the case of each mineral check reasonably well. It is also evident that r for the Keystone mineral is a little more than ½ per cent. and for the Sinyaya Pala about ¼ per cent. It is to be noted also that the difference of age of these two minerals is roughly 4×10^8 years. Considering these results it would be desirable to have the experimental values of r from these minerals.

The following general conclusions about actino-uranium seem plausible:

(1) Actino-uranium seems to be an independent isotope of uranium.

(2) Its disintegration constant is about $\lambda_{ac\,u} = 2.5 \times 10^{-9}\ \text{yr}^{-1}$ or $T = 2.7 \times 10^8$ years.

(3) Its amount in minerals appears to conform to the idea that it is initially of a definite amount in proportion to the uranium and that this relative proportion decreases with the age of the mineral.

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ON THE MECHANISM OF BIOLOGICAL OXIDATION AND THE FUNCTION OF THE SUPRARENAL GLAND

Clinical and experimental observations on the symptoms of suprarenal deficiency have indicated that the suprarenal gland is in some way involved in the mechanism of oxidation. For this reason I have studied for many years processes of oxidation, with the hope of finding the clue to the function of the cortical part of the gland. Vegetable tissues were used in these studies to a great extent.

Most of the common plants and fruits can be divided into two main groups: those which show discoloration on injury (apple, banana, potato), and those which do not (lemon, orange, cabbage).

Extending earlier observations of Palladin and M. W. Onslow, it was found[1] that the most prominent oxidation system of tissues which discolor on injury is the so-called phenoloxidase system. This system consists of a phenoloxidase and a phenol, which latter is a derivative of catechol and is chemically closely related to epinephrin. In the presence of molecular oxygen, this phenol is oxidized by the phenoloxidase to an ortho-quinone. In the intact tissue, this quinone is rapidly reduced by hydrogen, which is derived from the foodstuffs. If the tissue is injured, this reduction does not take place; the quinone remains in the oxidized state and undergoes secondary changes, or combines with other cellular constituents. The quinones and their secondary products are highly colored and thus cause discoloration of the tissue.

It is known that quinones have a very high bactericidal power. It has been shown in the latest experiments on potatoes that injury to the tissue leads to increased oxidation of the phenol and therefore to increased production of quinones, so it seems probable that the phenoloxidase systems also play a part in the natural immunity of plants which contain this oxidation system.

Most vegetable tissues which do not have the phenoloxidase system and which do not discolor on injury contain a highly active peroxidase and very little catalase. Further investigation has shown that these tissues also contain, in relatively high concentration, a substance which is characterized by its strong reducing properties. This substance has been isolated in crystalline form and has been found to be a hitherto unknown compound related to the carbohydrates. This acid is an isomer of glucuronic acid and has been named "hexuronic acid." This acid shows quite unexpected activity. It reduces salts of silver even in the cold and in an acid solution. It is the most reactive derivative of the carbohydrates hitherto discovered and prepared in free condition.

The most striking property of this substance is its reversible oxidizability, by which two of its hydrogen atoms can be split off in a reversible way. The substance owes its high reactivity to these two labile hydrogen atoms, and not to the carbonyl group.

The latest studies performed on cabbage leaves have brought out the fact that in this plant hexuronic acid plays a central rôle in normal respiration. It has been shown that the cabbage leaf contains a very active enzyme which oxidizes hexuronic acid at a high rate, oxidizing off its two labile hydrogen atoms. The oxidized hexuronic acid is then again reduced. In this way, the hexuronic acid connects as a hydrogen car-

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[1] A. Szënt-Györgyi, "Über den Oxydationsmechanismus der Kartoffeln," *Biochem. Ztschr.*, 162: 399–412, 1925.