Section of crystal	Lead Per cent.	Uranium Per cent.	Thorium Per cent.	$\frac{Pb}{U+0.36\,Th}$	Approximate age in million years
Outside layer	9.74	37.85	8.36	0.238	....
Middle layer.	11.93	58.48	14.09	.188	1,427
Core	11.87	60.70	8.05	.187	1,418
Whole crystal	11.13	47.73	9.55	.218	....

From the results it seems that, in the long process of alteration, uranium has been removed faster than lead. This is in agreement with the general belief that ages based on analyses of altered minerals are too great.

A study of the composition of the two inner layers, both of which were apparently unaltered, and certainly were both of the same geologic age, is most interesting. The lead content is nearly constant. The fact that the thorium content of the core is considerably less than that of the middle layer is surprising. It is possible that, during the formation of the crystal which probably required only a comparatively short time, the concentration of the thorium increased in respect to uranium. If that be true then the low value for thorium in the outside layer must be attributed to leaching.

The value of the "k factor," which converts the thorium content to an equivalent amount of uranium in lead-producing capacity, must be such that the ratio calculated for minerals of the same age but with varying amounts of uranium and thorium will be the same. Since the thorium and uranium contents are not the same in the two inner sections of this mineral, all of which must be of the same approximate age, the value of this "k factor" can be calculated from the analytical data.

$$\frac{Pb\ (middle)}{U\ (middle) + k\ Th\ (middle)} = \frac{Pb\ (core)}{U\ (core) + k\ Th\ (core)}$$

Substituting the values for Pb, U and Th from the table, k is found to be 0.42. This is somewhat higher than 0.36, which is commonly accepted as the most probable value for this constant. Due to the lack of proof that alteration has not affected the composition of these inner layers, little weight can be given this calculated value of k.

It has been suggested by Professor Alfred C. Lane[1] that, if, during the formation of the crystal in the pegmatite, there was a transfer of uranium into veins where we find it as botryoidal pitchblende uniformly free from thorium, we might expect a greater proportion of thorium in the middle layer of the crystal, and this would, perhaps, account for the higher k factor and slightly higher lead ratio than was found for the much larger crystal of Wilberforce uraninite analyzed by Wells and used by Baxter and Bliss[2] for atomic weight determinations of lead.

[1] Private communication.

CONCLUSIONS

From the data at hand, the following conclusions are suggested.

In the alteration of uraninite, lead is lost less rapidly than uranium and thorium.

In order to calculate accurately the age of a mineral by the so-called lead method, it is necessary to have analytical data on fresh, unaltered specimens.

Since most uraninites and pitchblendes are altered to some extent, the calculated ages are likely to be too great.

The Th/U ratio in different zones of a single crystal of uraninite may vary. This, in the case of unaltered uraninites, may be due to a change in concentration, during the growth of the crystal, of these elements in the medium in which the crystal was produced.

Assuming no alteration in the two inner zones of the specimen analyzed, the lead-uranium ratio leads to a value of about 1,420 million years for the age of this mineral. This is only slightly higher than the results of other investigators for the age of Wilberforce, Ontario, uraninite.

A method is suggested for calculating the relative lead-producing power of uranium and thorium from the analytical data on sections of single crystals of minerals in which the Th/U ratio varies, due to some other process than alteration.

Further study of the effect of alteration on the lead ratio of radioactive minerals is in progress by one of the authors.

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APPARENT INTOXICATION IN POULTRY DUE TO NITROGENOUS BASES

DURING a series of experiments upon commercial protein supplements we observed a condition in poultry which we believe to be an intoxication induced by nitrogenous bases. The diet upon which the symptoms were observed contained yellow corn 64, wheat 20, functionally low sulfur fish meal 10, dried yeast 1, calcium carbonate 1.1, tricalcium phosphate 0.4, and sardine or cod liver oil 1 part. It will be noticed that this ration is similar to the one employed by Holst and Halbrook[1] in their studies upon "scurvy-like" disease in chicks, with the exception that the concentration of the protein supplement is here within normal limits for growing chicks.

[2] Baxter and Bliss, *Jour. Am. Chem. Soc.*, 52: 4851, 1930.

[1] W. F. Holst and E. R. Halbrook, SCIENCE, 77: 354, 1933.

The general characteristics of the syndrome can be described as follows: (1) There was an anemia which in some cases was so severe that there remained less than 1.7 gm hemoglobin per 100 cc blood; (2) the clotting time of the blood was greatly lengthened; (3) the blood picture was distorted and was generally characterized by the presence of (a) immature red cells, (b) a high white count, which tends to become abnormally low if the bird becomes moribund as the result of weakness and ceases to eat or drink, (c) a fall in the percentage of polymorphonuclear leucocytes and a corresponding rise in the percentage of lymphocytes; (4) the striking occurrence of hemorrhages without respect to location. However, hemorrhages are not always observed upon birds of this type, even though the other symptoms may be present.

In order to obtain a hemorrhagic bird it must be placed upon an appropriate diet at an early age. When birds are placed upon a normal ration for the first few weeks of life, we have not been able to induce hemorrhages by subsequently feeding a hemorrhagic ration, although the blood picture may be as described above. This observation with respect to the occurrence of hemorrhage may explain the inability of Cribbet and Correll[2] to produce hemorrhagic symptoms in chicks with the Holst-Halbrook ration, since they did not begin to feed such a ration until the chicks were 25 days old. We have been able to produce the symptoms at will during the past two years, provided the birds were placed upon the hemorrhagic diet when 7 days of age, or younger.

A similar set of symptoms, including hemorrhages and low hemoglobin but not including the blood picture, was described by Holst and Halbrook as a scurvy-like disease, and they were of the opinion that it was due to the lack of vitamin C. Since scurvy-like symptoms may be induced by many liver poisons, such as fluorides, phosphorus, benzene, alkylamines and ergot, the occurrence of such symptoms does not necessarily indicate a lack of vitamin C. The hemorrhagic ration of Holst and Halbrook contained approximately 20 per cent. fish meal, and it is stated by them that a decrease in the percentage of fish meal by the addition of 10 per cent. dried skim milk produced almost normal chicks. Since it is extremely doubtful if dried skim milk contains any vitamin C,[3] it is hard to understand why the beneficial results appeared if the primary difficulty was due to the lack of vitamin C. Since we are able to show that at least a portion of the symptoms are due rather to the presence of nitrogenous bases in the functionally low sulfur fish meal fed, a more plausible explanation lies in the fact that fish meal concentration was lowered and in that manner the intake of nitrogenous bases was decreased.

A similar set of symptoms has been reported by Dam,[4] in this case induced by a diet composed of vitamin-free casein, marmite, cod liver oil concentrate, corn-starch and salts. However, the pathological symptoms of these birds as reported by Schönheyder[5] do not include morphological changes in the blood. Since we have always found a distorted blood picture whenever nitrogenous bases were administered and not detoxified, it is possible that we are dealing with both the anti-hemorrhagic factor as reported by Dam and in addition the effects of materials such as the nitrogenous bases.

The results of our investigation show that with a diet such as the one we used, the replacement of functionally low sulfur fish meal by meat scrap, casein, skim milk, or functionally high sulfur fish meal resulted in a definite decrease in hemorrhagic symptoms. We also observed that the addition to an otherwise non-hemorrhagic diet of trimethylamine, dimethylamine, monomethylamine, diethylamine, dipropylamine, isoamylamine, ergot and nicotine gave a set of symptoms similar to that produced by the hemorrhagic diet described above. We are also able to demonstrate quantities of bases, mainly methylamines, in composite samples of functionally low sulfur fish meal and in fresh specimens of the fish from which the meal was made which would be sufficient to produce the intoxication in the diet fed. Methylamines were also present in the functionally high sulfur fish meal, but owing to the nature and amounts of the sulfur compounds contained therein, detoxication was possible.

In looking for some method to counteract the presumed effect of the nitrogenous bases, we investigated the possibility of combining these substances with a haem compound in the presence of a reducing agent. (For it has been known since the work of Barcroft, Anson and Mirsky, and others, that compounds of this type combine easily with hemoglobin to form hemochromogens). When we added dried blood and certain sulfur-containing reducing agents (particularly elemental sulfur, cystine and cysteine) to hemorrhagic diets, such as those containing functionally low sulfur fish meal, alkylamines and ergot, a marked alleviation of symptoms was observed.

This investigation is not yet complete, but the data indicate that diets including such substances as certain of the nitrogenous bases may be detoxified by the administration of materials rich in sulfur-containing amino-acids or reducing agents contained in or derived from plant or animal materials plus some haem compound, if it is not otherwise present.

[2] R. Cribbet and J. T. Correll, SCIENCE, 79: 40, 1934.

[3] E. B. Hart, H. Steenbock and N. R. Ellis, *Jour. Biol. Chem.*, 46: 309, 1931.

[4] H. Dam, *Nature*, 135: 652, 1935.

[5] F. Schönheyder, *Nature*, 135: 652, 1935.

Further work is in progress, and the entire investigations will be reported in detail elsewhere.

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THE DIPHASIC ASPECT OF THE CURDLING OF MILK BY RENNIN

STUDIES of rennin action are all based upon the physical changes which it induces in milk or casein solutions. The end-point of the process is the appearance of a flocculent precipitate, the formation of a solid gel or the rise in viscosity immediately preceding these gross evidences of rennin activity. Added ions or compounds are found to accelerate or retard the process as a whole, and this is usually interpreted to indicate acceleration or inhibition of the enzyme. As a matter of fact, we may have marked change in curdling time without any change in the activity of the enzyme. It is clear that the curdling of milk involves two distinct phases. One is the enzymic phase, in which, according to Bosworth, casein is split into two molecules of paracasein. The other is the phase of aggregation, in which paracasein separates out as a precipitate or forms a gel. The two phases may be separated for the purpose of study in the following way.

To a standard milk preparation a small amount of rennin is added. Samples are removed at intervals; treated with formaldehyde to check the enzyme, and with sufficient calcium chloride to precipitate paracasein only. The volume of the paracasein is measured in a graduated centrifuge tube after whirling at a given speed for a given time. Nitrogen determined on the supernatant indicates accurately the residual casein left in the digest at the time of sampling. When plotted, the curves of paracasein increase and casein decrease are reciprocal. When paracasein reaches a constant and maximum volume, the supernatant liquid is perfectly clear and shows no casein N. This marks the end of the enzymic phase. The phase of aggregation ends when the paracasein precipitates or curds. The enzymic phase occupies less than 60 per cent. of the time required for the appearance of curd. From the data obtained, therefore, we may conclude that the digestion phase of curdling is over in about half the time required to develop the actual curd.

If calcium chloride is added to the milk, and a similar digest started, with accurate control of pH, the process as a whole is accelerated. When studied by the method outlined, it is found that the calcium salt has no effect upon the enzymic activity of rennin, but markedly shortens the time required for the precipitate or curd to form. On the other hand, an increase of the H ion accelerates both phases.

We expect to report later in more detail studies of the two phases involved in curdling of milk by rennin and other proteases.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TUBE FOR CULTURING FUNGI

IN connection with experimentation on the temperature and oxygen relations of growth and respiration in some wood-inhabiting fungi cultured on solid nutrient agar, a new form of culture tube has proved very satisfactory. It consists of the ordinary test-tube, modified by a rounded indentation or invagination of the wall on one side near the mouth, as shown in the accompanying diagram. This modification is

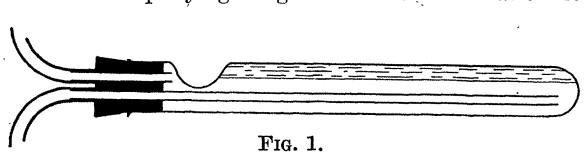

FIG. 1.

easily accomplished by holding the test-tube at right angles to the flame of a wing-top Bunsen burner until the portion of the wall which is to be altered is sufficiently softened, when gentle mouth suction is applied internally by means of rubber tubing attached to a bit of glass tubing that leads through a cork stopper set tightly into the test-tube opening. Finally, the heated portion should be subjected to a yellow flame to insure adequate annealing. With a suitable flame tubes of hard or other chemically resistant glass may be employed.

Such a modified tube is charged with agar substrate in the usual way, excepting that it is not slanted but is kept horizontal, with the indentation below, while the agar solidifies. Since the indentation prevents the escape of the agar while still liquid, there results a uniform narrow strip of solidified substrate (shaded in the diagram) lying along one side of the tube. The tube when thus prepared can be used for short-term growth studies with advantages noted by Fawcett in connection with his long-tube cultures of parasitic fungi.[1] Visible characteristics of growth may be studied by means of surface observations, or inspection through the substrate if the latter is reasonably

[1] H. S. Fawcett, *Ann. Appl. Biol.*, 12: 191–198. 1925.