tion of the mean pathways for disturbances of acid-base balance (*9*). It promises to reveal additional insight into acclimatization to altitude and into the various stages of compensation to acid-base balance disturbances.

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Technical Papers

Radioactivation of Colloidal Gamma Ferric Oxide

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Welo and Baudisch (*7*) were the first conclusively to establish that ferric oxide—Fe_2O_3—exists in both a cubic and a rhombohedral form. The cubic form, γ ferric oxide, is the labile one and ages slowly, under loss of energy, into the stable rhombohedral form, the common α Fe_2O_3 or hematite. The cubic or spinel type, γ Fe_2O_3, possesses magnetic properties similar to, but feebler than, metallic iron and, in addition, a remarkable number of catalytic and biocatalytic qualities. The rhombohedral, α Fe_2O_3, which is not magnetic, is also active in a biocatalytic manner but to a lesser degree than the cubic form.

It is obvious that the difference in physical behavior of the two chemically identical modifications of iron oxide must be attributed to the difference in their crystal structure. Many investigators have studied the crystal structure of these iron oxides (*6*). The result of these investigations was the discovery that the lattice in the structure of γ Fe_2O_3 is incomplete. The lattice of γ Fe_2O_3 contains so-called "interstitial spaces" (atomic holes) which, in the more stable lattice of α Fe_2O_3, are filled up by ferric ions. The elemental crystal of γ Fe_2O_3 contains an average of $21\frac{1}{3}$ ferric ions compared to an average of 24 found in the crystal of α Fe_2O_3. This was ascertained mainly by a number of experiments dealing with the diffusion of liquids and gases in these crystals. It was found, for instance, that radium emanation diffuses freely through the "atomic holes" in γ Fe_2O_3 crystals, while α Fe_2O_3 is almost impenetrable to this emanation; in this case, the "atomic holes" are blocked at room temperature. The diameter of these "atomic holes" or channels in γ Fe_2O_3 ranges between 5.2 A. and 7.5 A., as the benzene molecule (diameter, 5.2 A.) may penetrate the crystal, but the xylene molecule (diameter, 7.5 A.) is unable to do so.

The fact that the cubic, ferromagnetic iron oxide possesses these "atomic holes" is of great significance, since within the crystal a secondary structure, or "inner surface" is formed. The comparatively great surface of this crystal is mainly responsible for the high catalytic reactivity of the material. Also, the "atomic holes" create electric disturbances within the crystal which may also influence the reactivity of the compound.

The properties of γ Fe_2O_3 remain unchanged when put into a colloidal state (*1*), and it is in this form that it is especially suitable for certain biological purposes. Colloidal solutions were prepared with a colloid mill, using as a carrying medium dextrin, olive oil, and gum arabic; the particle size obtained was 10^{-5} cm. or smaller.

Gamma ferric oxide in colloidal form may be injected directly into the blood stream. Peyton and Beard (*5*) found that the colloidal particles of γ Fe_2O_3 are taken out of the blood stream by the reticulo-endothelial cells, which are phagocytic to foreign materials of this type. These authors first isolated Kupffer's cells (reticulo-endothelial cells of the liver) by passing a sodium chloride solution through the liver and then using an electromagnet to separate the cells, the latter having been transformed into living magnets by the absorption of colloidal γ Fe_2O_3 particles. These reticulo-endothelial cells are distributed throughout the body, but are most abundant in the liver, spleen, and bone marrow. They

play an important role in the case of chronic inflammations, in the repair stage of acute processes, and in blood diseases. Colloidal gamma iron oxide has no toxic effects on the living cells; the cells proliferate and, after some time, have eliminated and are entirely free of all the iron particles.

It is easily seen that the reticulo-endothelial cells in the body can be influenced and their antibody actions stimulated if the colloidal γ Fe_2O_3 particles are combined with some therapeutically acting material, such as certain radioactive substances. A method of doing this is the main subject of the present article.

Experimental Evidence

It was first thought that radon gas (radium emanation) might be used as the radioactive substance with which to activate the colloidal γ Fe_2O_3 particles, but this proved to be impractical, since radon cannot be stored in this type of crystal because of the "atomic holes" in the lattice (which are responsible for the good qualities of γ Fe_2O_3 as an emanator). The idea of using radium itself as the activating material had to be discarded, as injections with Ra are not permissible. This also was the objection to the use of polonium, which is highly toxic unless used in infinitesimal quantities.

It was therefore necessary to look elsewhere for a radioactive substance which would be an alpha radiator, which could be deposited easily and firmly on and within γ Fe_2O_3, and which had a short half-life in order to avoid dangerous aftereffects.

The active deposits of radon—Ra A, Ra B, and Ra C—were finally chosen as activators. Ra A is an alpha-ray emitter with such a short half-life (3.05 minutes) that its activity, in this case, is negligible; Ra B, with a half-life of 26.8 minutes, is a beta- and gamma-ray emitter; and Ra C, with a half-life of 19.7 minutes, is the most potent alpha and gamma radiator in the radium series. Depositing Ra A, Ra B, and Ra C in equilibrium conditions onto another substance, Ra C (the substance in which we are predominantly interested for our experiments), would decay approximately with the half-life period of Ra B.[1]

Experiments were then made with the object of finding a suitable method of deposition of the radioactivating substances on and within the colloidal γ Fe_2O_3. Generally, activations with the active deposits of Ra emanation are done by an electrical method. The substance to be activated is exposed to the emanation in an electrical field, in which the substance itself has to serve as the negative electrode. This method, although feasible in our case because of the metallic properties (conductivity) of iron oxide, which means that it could be used directly as the negative pole, was not employed, because the yield of such an exposure is small and the active deposit does not adhere very firmly. In addition to the preceding objections, it would only be possible to activate the surface layer of the exposed colloidal powder, an area which would be entirely insufficient in our case.

[1] Ra C is formed by the decay of the longer-life product, Ra B, and will disappear approximately with the decay of Ra B.

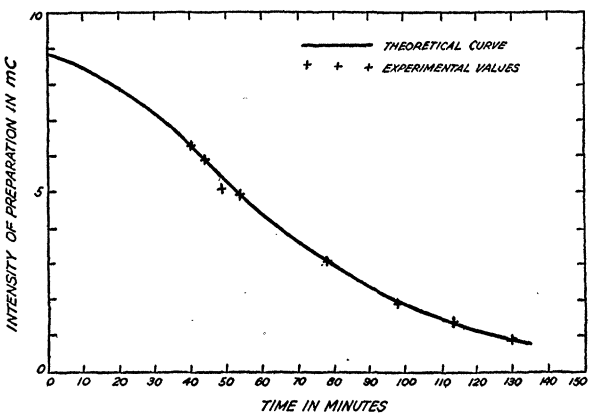

Fig. 1

For the above reasons another method of activation, similar to that first used by H. Petterson (*4*) for the activation of metallic disks, was adopted.

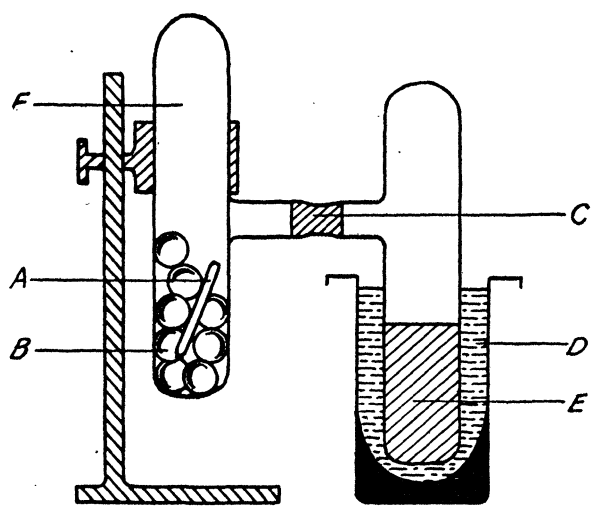

Fig. 2. A—glass capillary enclosing Ra emanation; B—metal shot; C—filter; D—liquid air; E—colloidal γ Fe_2O_3.

The pyrex glass apparatus shown in Fig. 2 was used for the activation of our compound. Into Tube E a small quantity (approximately 5 grams) of colloidal γ Fe_2O_3 powder was introduced, and the top of the tube sealed hermetically. The powder was then heated to about 150° C. while evacuating the apparatus through Tube F, in order to eliminate all possible gases that may have been absorbed in the powder and

which might clog the atomic channels within the crystals. Care was taken to keep the temperature at 150° C., since too high a temperature would result in the transformation from gamma to alpha iron oxide. This too may be controlled by testing the colloidal material for its magnetic properties (absent in α Fe_2O_3) after the initial heating.

Tube F contains a thin glass capillary which in turn encloses a known amount of Ra emanation. Tube F also contains a few metal shots.

After the powder in Tube E has been sufficiently heated, Tube F is sealed, care being taken to maintain the vacuum in the system. The apparatus is then shaken mechanically until the shot contained in Tube F has broken the glass capillary and freed the emanation.

The radon is now distributed equally throughout the apparatus, so that only part of the radioactive gas is in contact with the colloidal powder. It is possible to concentrate Ra emanation onto another substance by freezing. The temperature required for this is rather low, and liquid air had to be employed as a cooling agent. The part of Tube E containing the colloidal γ Fe_2O_3 was immersed in liquid air so that the radon might freeze upon the powder. The emanation concentrated in this manner has to remain in contact with the powder for three and one-half to four hours until equilibrium is reached between the emanation and its active decay products.

Taking into account the atomic channels of γ Fe_2O_3, it is evident that the emanation will have close contact not only with the surface but also with the interior of the crystal lattice of the powder particles. After allowing the necessary time for the attainment of equilibrium, the apparatus is broken, and the remainder of the emanation present is discarded. The powder is again heated (again the temperature is to be maintained at less than 150° C.) to drive off any emanation that may be present in the channels of the powder or particles. The active deposit—Ra A, Ra B, Ra C—adheres very firmly to the powder as it is hammered onto the particles by two successive alpha disintegrations. The colloidal γ Fe_2O_3 powder may now be dispersed in any liquid medium suitable for injection therapy.

In order to establish a definite method of dosage, the activity of the powder has to be measured before injection. Taking these measurements at different time intervals assures that we are really dealing with Ra B and Ra C and provides a definite check as to whether or not all the emanation is evaporated from the powder.[2] How completely this purpose was accomplished may be seen from the points plotted on the decay curve of Ra C (Fig. 1). The points on the curve correspond to the values of Ra C obtained when measured by its gamma radiation. The curve was drawn in accordance with the theoretical values for the decay of Ra B–Ra C after four hours exposure in decaying radon. The starting point of the curve and the absolute values of the ordinates are extrapolated from experimental values for the moment at which the emanation was discarded. The fact that all the experimental points are very close to the curve proves that no radon remained in the colloidal powder and that the extrapolation to the zero point is correct. This zero point, which gives the activity of Ra B–Ra C reached by the exposure in radon, is a measure for the yield of the above process of activation.

[2] Presence of radon within the powder not only would falsify the dosage required for the treatment but also might be dangerous if used in large quantities.

Various other experiments, using γ Fe_2O_3 of different particle sizes, were made with the result that the best yield was obtained, as expected, with the material in colloidal state. Without taking any special precautions, a yield of 90 per cent was easily obtained. Certain of the experiments performed in order to ascertain the preceding may be of interest.

The behavior of powdered γ Fe_2O_3 was compared with that of colloidal γ Fe_2O_3, using the type of apparatus described above but adding an extra tube in order to have similar conditions for the two compounds used. About 15 times as high a yield was obtained for the γ Fe_2O_3 powder as for the colloidal γ Fe_2O_3, although the surface of the colloidal powder is much larger. The reason for this was later found to be the following: The colloidal γ Fe_2O_3 was prepared with mineral oil as a carrying medium. The mineral oil was later removed with petrol ether and the solid colloidal γ Fe_2O_3 heated to 150° C. Traces of the oil were, however, not completely evaporated, since care was taken not to exceed the 150° C. temperature mark. This oil clogged the atomic channels and prevented the radon from penetrating within the lattice of the powder particles. Although mineral oil has, at ordinary temperatures, a strong adsorption for radon, this seems not to be the case for very low (liquid-air) temperatures, at least not in comparison with the adsorption coefficient of Fe_2O_3.

Another experiment was undertaken in order to differentiate the adsorption factors of gamma and alpha ferric oxide. In order to have the same colloidal conditions present throughout, we used the same colloidal γ Fe_2O_3, transforming half of the amount into α Fe_2O_3. This was done by heating the γ Fe_2O_3 to about 400° C., after which the powder showed only a very slight magnetic reaction, proving that at least 95 per cent of the total amount had been transformed from the gamma into the alpha modification. The

three-tube arrangement on our apparatus was again used to make possible a full comparison of the results. It was found that the yield in amounts of Ra B–Ra C adsorbed was from 40 to 50 per cent higher in the case of γ Fe_2O_3, calculated for equal quantities of powder. As the formation of the active deposit depends upon the adsorption of emanation (all adsorption phenomena are surface effects), this result means that the "inner surface" of the γ Fe_2O_3 powder in colloidal state is from 40 to 50 per cent greater than the surface of the alpha modification. Taking into consideration this great surface of colloidal γ Fe_2O_3, we can now understand why this material, in the colloidal state (average particle size, 0.1 μ in diameter), has such great catalytic efficiency.[3]

Application

We believe that there is no limit as to the charge of Ra B–Ra C that may be applied with the colloidal γ Fe_2O_3. The quantity of Ra B–Ra C desired can be easily obtained, depending only upon the initial quantity of Ra emanation used and upon the time elapsing between the discarding of the emanation from the powder and its injection into the blood stream. In order that the powder may have sufficient activity when used for injection therapy, it may remain in contact with the emanation until the last moment and so may be shipped to distant places if the part of the apparatus containing the powder is kept at a low temperature by either liquid air (in a thermos flask) or dry ice (solidified CO_2). It was found that when dry ice (– 60° C.) is used, approximately 50 per cent of the emanation may be fixed to the powder. The quantity of Ra B–Ra C to be used in the treatment may be calculated in advance as long as the initial quantities of radon, the time of storage in the apparatus or shipping, and the time elapsed between the opening of the apparatus and the application of the powder are known, so that an exact dosage is possible at all times.

An important advantage of colloidal γ Fe_2O_3 lies in the fact that it may be suspended in an aqueous medium and so be used for intravenous injections. The activated iron oxide powder is an alpha, beta, and gamma radiator; injected into the blood stream, it enters (as already mentioned) the reticulo-endothelial cells. The alpha particles emitted by the powder will be absorbed, almost entirely within the cells, all the energy being transmitted to the cells. The amount of beta and, especially, gamma radiation absorbed in the same cells is negligible in comparison with the alpha radiation. Not even 1 per cent of the ions formed in the cells will be due to beta radiation; and the number of ions produced by gamma radiation is only about 0.01 per cent of those produced by alpha particles. For that reason we can suppose that within the cells only the alpha radiation is biologically active; the tissue near the cells will receive beta radiation, while the action of the gamma radiation is spread over the entire body. Because of the strong and concentrated action of the alpha particles, however, we can limit the intensity of our preparations to very low values, so that the effect of beta and gamma radiation is of no importance.

If using preparations of 1 mc. (Ra B and Ra C), we have within the cells, by alpha action, ionization effects which with the common Ra therapy could be produced only with a preparation of 10 grams of Ra.

It is to be presumed that the highly activated reticulo-endothelial cells will be more effective in their action against bacteria and inflammatory diseases. These cells are distributed throughout the body, but are found especially in organs connected with the blood formation.

Leblond and Lacassagne (*3*), using subcutaneous injections of polonium in experiments with rats, proved by autographic methods the presence of polonium within reticulo-endothelial cells upon the lymphatic formations and also found a pronounced effect upon the lymphatic system. They recommend the use of polonium for the treatment of lymphatic leukemia. However, since polonium is toxic and produces gastric ulcerations, it would be desirable to replace it by activated γ Fe_2O_3, as described above. The use of the colloidal gamma iron oxide activated with Ra B and Ra C is preferable even to artificial radioactive gamma iron oxide (beta radiator), since in the latter case relatively high activities have to be used in order to produce the same intracellular effects; also, artificially radioactive iron has a relatively long life (47 days half-time), so that strong sources may be dangerous. On the other hand, in the case of activation with Ra B and Ra C, no dangerous after-effects have to be considered. One-tenth mc. of Ra B, after its complete decay, forms 2.5×10^{-6} mc. of polonium, if none is eliminated, so that even if we repeat the treatment from 10 to 20 times, we are still far below the amount of polonium formed by 0.1 μg. of Ra, the generally accepted tolerance dose.

It may, however, be even more advantageous to use, instead of Ra B–RaC, the active deposit of thorium, Th B and Th C. Th B has a longer life (10.6 hours half-time), and Th C also is the last radioactive element produced in this series, as Th D is already a stable element.

[3] We want to point out that this method is probably the simplest and most exact for the determination of grain sizes. Comparing it with the diffusion method, we find that we have no correction factors that may multiply any potential errors. The real surface of the powder, or grains, is simply given by the activity of the powder in comparison with that of the cooled glass tube, the surface of which can easily be measured.

In this connection it is interesting to refer to a recent paper of Kelsall (*2*), who emphasizes the relationship between lymphocytosis and cancer. If we reduce by a radiotherapeutic method the number of circulating lymphocytes, there is some hope to reduce also the growth and occurrence of tumors.

The activated gamma iron oxide (Ra B–Ra C or Th B–Th C) is interesting not only for therapeutic use but also from a biological point of view.[4] We could, for instance, follow from the outside the distribution of the activated iron oxide with a Geiger counter while trying, by means of a strong magnetic field, to influence the path of the activated magnetic cells (*1*).

Finally, using the method described by Rous (*5*) for separating Kupffer's cells by magnetic methods, the way would be open to a study of the influence of alpha particles on living cells.

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The Age Factor in Adaptability of a Sarcoma Virus to Other Animal Species

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An indispensable condition for the adaptation of avian sarcoma viruses to alien species of birds is the youth of the individual in which infection is attempted, this culminating in the case of ducks, which respond to the inoculation of viruses of the Rous and Fuginami sarcomata for only 24 hours after hatching (*3, 4*). The results that follow show that the age of the chicken bearing the tumor is also a factor of importance in determining the adaptation of the virus of the Rous sarcoma to ducks.

[4] An interesting paper on this topic was published by Maxfield and Mortensen (*J. appl. Phys.*, 1941, **12**, 197), who used, in experiments with rats, colloidal thorium dioxide. They found, by radioactive measurements, that six to eight hours after an intravenous injection, 99 per cent of the thorium was removed from the blood stream by the phagocytic action of the reticulo-endothelial cells. They recommend the use of this method for tests of the various theories of phagocytosis. However, using this method on man may be dangerous because of the aftereffects that are due to the long-life, daughter-product, mesothorium.

The experiments consisted of the inoculation into the breast of newborn ducks of cell suspensions from tumors grown in chickens varying in age from 15 days to 18 months, and of analogous inoculations into successive groups of ducklings of the tumors obtained in the preceding passages at intervals of from 1 to 3 weeks. In some cases, additional ducklings were injected in the vein with tumor filtrates, and the growths ("late tumors") that evolved several months thereafter were transferred to other groups of ducklings by means of cell suspensions.

The following results were obtained:

(1) The tumors grown in chicks from 2 to 4 weeks of age were very easily transferable into ducklings in 16 out of 17 lines, each started from a different chick tumor. Growth was fast, and the hosts often died although the tumors never became generalized. Transplantation of these tumors was successful, although irregularly, for a number of passages which, however, never exceeded 6, and always in the absence of generalization. The number of ducks developing tumors became progressively smaller in the course of the passages until none of the animals responded. The tumors in the surviving birds regressed in every case, and as a result, all the lines were lost, despite the fact that a total of nearly 350 ducklings were inoculated. Despite its long sojourn in a foreign species the virus did not show detectable signs of variation, since filtrates from the duck-grown tumors produced the customary lesions at the usual rate in chicks.

(2) The tumors grown in chickens 18 months of age did poorly in ducklings for in 6 of the 8 lines started, growth followed in only 3 out of 47 birds injected, and these two tumors could not be carried beyond a second passage. However, in the two other lines growth followed in 8 of the 11 ducklings injected, and adaptation was achieved after some passages with the customary succession of events accompanying the phenomenon.

(3) The tumors grown in chickens intermediate in age between the two above groups seemed to be the most adaptable to ducks. Most of the chickens of this group were from 5 to 10 months old, while another also supplying an adaptable tumor was 3 months old. Of the 6 duck-tumor lines which have been obtained, 4 were evolved by passages of cell suspensions, while in the other 2 cases ducklings were successfully injected with both cell suspensions and filtrates, but the lines were obtained by passages of the "late" tumors induced by filtrates. To these cases one has to add at least 3 more in which filtrates induced typical "late" tumors, but neither these growths nor those produced by cell suspensions were passed into other ducks. However, adaptation was not