IN THE LABORATORY

Apparatus for Studying Crystal Formation

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In an attempt to develop methods for studying some of the problems of mineral deposition, we have designed a simple apparatus which promises to be very useful in many different kinds of studies of crystal growth. Most recorded laboratory experiments on crystallization have made no attempt to simulate the conditions under which many crystals are formed in fissures and cavities in the earth. One of the most common mechanisms under natural conditions is precipitation by slow cooling from moving solutions. The apparatus is a first attempt to design an approximate earth model in which the effect of variables can be studied accurately.

The essential features of the apparatus are shown in Fig. 1. The material to be precipitated is ground, sized, dried, and weighed, then put in the pervious basket in the left arm above

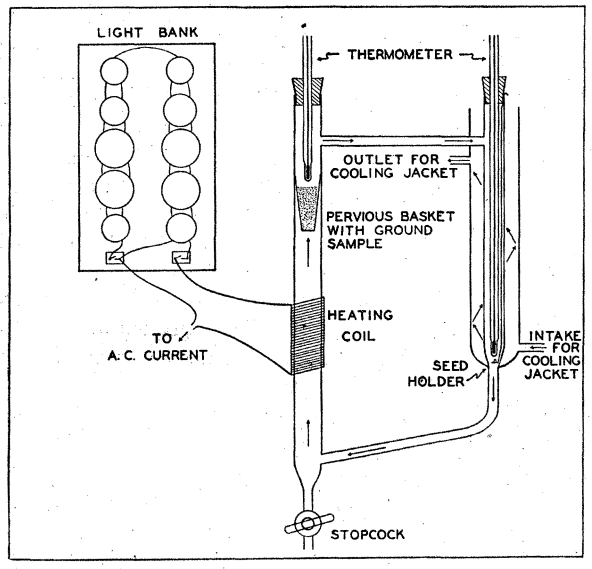

FIG. 1

the heating coil. The heating coil is connected, and convectional circulation established as shown. The temperatures of the two arms can be controlled by adjusting the amount of heat by using various bulb combinations in the lamp bank and by adjusting the rate of flow of cooling water in the jacket surrounding the right arm. After a constant temperature gradient has been established and the solution is saturated at the lower temperature, a seed crystal is introduced at the coldest point of the right arm, and growth on the seed begins.

Many different kinds of studies can be made with the unit. Some of the obvious advantages over ordinary static precipitations might be mentioned. Operation is continuous, and the system is so stable that little adjustment is necessary after an original temperature setting. The apparatus is especially suited for studies of slightly soluble materials, since thousands of liters of solution can be passed through the sample. Preliminary work indicates also that many slightly soluble substances, which precipitate colloidally when formed by the mixing of ionic solutions, will crystallize fairly readily down a gentle temperature gradient such as that provided in the apparatus.

The device is now being used to study the effect of flow on crystal orientation and growth, the use of inclusions in crystals as a criterion of temperature of formation, and the rates of leaching of very slightly soluble minerals as a function of temperature. Many other possibilities suggest themselves, such as the response of crystal habit to different temperature gradients and the nature of replacement processes. A second model has been designed with adaptations to fit our specific purposes. This new model will have a plane-sided chamber at the point of crystal formation, so that the crystals can be photographed during growth and the prints pieced into a motion-picture film. In the first design, rate of flow and temperature differential of the two arms are interdependent, and the rate of flow changes with the grain size and total amount of material introduced for leaching. In the new design, rate of flow will be controlled by a diaphragm with adjustable permeability, so that flow and temperature differential can be kept constant, even though the permeability of the sample changes during the leaching process.

Antigenic Carbohydrate-Lipid Isolated From Paraffin-Oil Extract of Dead Tubercle Bacilli[1]

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A carbohydrate-lipid complex isolated from tubercle bacilli has been found to induce antibodies when injected into normal animals.

The experiments have been carried out, using as tools the two fractions of biological significance which were obtained from a paraffin-oil extract of dead tubercle bacilli (*1*). One fraction is called the "toxic" fraction, since as small an amount as 2 γ is sufficient to produce lesions in the lung of normal guinea pigs when injected intraperitoneally in paraffin oil. The other is called the "sensitizing" fraction, as it gives to normal animals definite hypersensitivity to old tuberculin (and also to the whole bacterial cells) when injected intraperitoneally, in oil, in amounts as small as 0.1 mg. Both fractions were found in the oil extract. This oil extract was made in an attempt to elucidate the mechanism by which paraffin oil enhances the ability of dead bacilli to produce

[1] This study was carried on under a grant from the Josiah Macy, Jr. Foundation.

hypersensitivity and lesions. We presumed, and we found, that the oil extracts these fractions from the bacilli. Both were precipitated from the oil with dioxane.

The "toxic" fraction is the chloroform-soluble portion of the precipitate after it has been thoroughly washed with dioxane and methanol. It is a polysaccharide ester of mycolic acid, which still contains, after purification, 1.05 per cent of nitrogen and 0.4 per cent of phosphorus.

The "sensitizing" fraction is that part of the precipitate which is insoluble in the usual organic solvents, such as methanol, ethanol, ether, chloroform, benzine, and petroleum ether. It contains a large amount of protein. (We found later, when we tried to purify these fractions, that each one of them is not free of the other.)

Preliminary experiments led us to believe that the "sensitizing" material acted also as a protective antigen. A clear-cut acquired resistance was obtained in normal guinea pigs which had been previously immunized by the "sensitizing" fraction and which were then injected with 0.1 mg. of living bacilli of low virulence, H-37.

In further experiments the normal guinea pigs were immunized with a *more purified* "sensitizing" material. The hypersensitized animals were then given bacilli of *higher* virulence (H-160 Corper). They showed a certain degree of acquired resistance, not only in survival times but also by the degree of tuberculosis involvement of their organs. But in spite of the fact that in four successive experiments, each one involving at least 15 sensitized animals and 15 controls, we lowered the infecting dose to 1/50,000 mg., these animals failed to show the same degree of acquired resistance as that shown by the animals which were sensitized by the less purified material.

It was clear that something else in the "sensitizing" fraction than the sensitizing antigen might be responsible for the previously observed acquired resistance.

On the other hand, animals which were injected with the "toxic" material alone in oil showed an excellent acquired resistance against infection when they received living bacilli more than three months after they were injected. The effective dose of "toxic" fraction was 1 mg. in one single injection, or 2 γ in two successive injections at an interval of a few weeks.

Moreover, there was some evidence that the "toxic" material, the carbohydrate-lipid component, produced antibodies. Guinea pigs immunized with this "toxic" material, as well as animals infected with tubercle bacilli, reacted more to the intracutaneous injection of a few gamma of the "toxic" material than did normal controls.

These observations led us to believe that the acquired resistance developed in guinea pigs by the "sensitizing" material was due to contamination of this material with the carbohydrate-lipid complex.

Our speculations received considerable support when we observed that the water-soluble portion of the hydrolysate[2] of our carbohydrate-lipid complex reacted strongly, in precipitin tests, with the sera of rabbits and guinea pigs which had been injected with the "toxic" carbohydrate-lipid complex alone. Precipitations were obtained in dilutions as high as 1:10,000,000 with some sera. Strong precipitations were also obtained with the sera of a horse which had been immunized with whole tubercle bacilli and also with the sera of rabbits that had been immunized with human and avian tubercle bacilli, grown in the "tween 80" medium recently described by R. H. Dubos and B. D. Davis (*2*).

This is the first demonstration that a chloroform-soluble carbohydrate-lipid complex isolated from tubercle bacilli is antigenic, when injected into normal animals in paraffin oil. Our experiments showed that this carbohydrate-lipid complex aids the process of acquired resistance to the tubercle bacillus. Whether or not this complex is the essential immunizing antigen of the tubercle bacillus remains to be seen.

In any case, it will be a useful tool—a test—which may allow us to follow, *in vivo* as well as *in vitro*, the carbohydrate-antibody formation in the course of infection with the tubercle bacillus.

[2] Hydrolysis made in methanol 10 per centpotassium hydroxide.

References

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A Technique for Obtaining Quickly Permanent Mounts of Nonembedded Botanical Material

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Plant pathologists and botanists are often embarrassed by being unable to preserve permanently material that has not undergone the classical technique of paraffin embedding. The former, for instance, of which the writer is one, would often wish to keep permanent mounts of such material as leaf scrapings or small fragments of bark, fruit, or freehand sections. These materials are usually preserved in a 7 per cent aqueous solution of potassium hydroxide. The mounts can be kept only temporarily even with asphalt lac seals.

As everyone knows, nothing can compete with balsam for permanent mounts, but with such material as is described above it is impossible to follow the classical procedure and, if this is not the case, one cannot always devote the time necessary for such inclusions.

The writer has adopted the following technique to circumvent the difficulty with most satisfactory results:

Slides that are worth keeping are first treated with chloralphenol (see M. Langeron. *Précis de microscopie.* (5th ed.) P. 607). This medium has the property of being miscible to water and balsam. Moreover, it is an excellent clarifying medium, and its miscibility to water makes it an excellent dehydrator. Droplets of this liquid are deposited close to the cover slip while the aqueous medium is pumped out with strips of filter paper. The slides are then slightly warmed to hasten the departure of air. The writer has found it convenient to lay them on the grating of a microscope lamp resistance. Levigation with chloralphenol is repeated until one can have the assurance that all the water is gone. Droplets of Canada balsam solubilized in xylol are then dropped on the site where chloralphenol has been previously deposited, the latter being in turn pumped out from under the cover slip with strips of filter paper. The slides are then heated at a slightly higher temperature to evaporate the chloralphenol. This must be