between the apparent object size and the width of the shadows produced by them were observed. In the case of rather small particles, such discrepancies were not regarded seriously and generally were explained by lack of contrast at the edges of the particles in the unshadowed specimens. Recently, however, such flagrant cases of discrepancy were observed that no such interpretation could be allowed. Fig. 1 reproduces a large particle with a shadow not only considerably wider than the particle but even extending a little

FIG. 1

toward the source in front of the particle. An examination of this and several similar micrographs excluded all interpretation other than a shrinkage of the particle *after* gold shadowing. The specimen in Fig. 1 consists of accidental impurities of unknown origin on a collodion film, which was gold shadowed in an evaporation apparatus and then transferred to the electron microscope. Apparently the particle size changed during observation (focusing) in the electron microscope and shows, therefore, a different appearance from that expected from its shadow. The observed shrinkage in this particular case is as much as 40 per cent. The question whether the particle changed previous to its gold shadowing is left hereby entirely open.

It is proposed, therefore, that in addition to such methods as comparison with other evidence (light microscopy, ultracentrifugation, streaming birefringence, etc.) as much evidence as possible should be gathered in the electron microscope about eventual structural or shape changes during observation. Gold shadowing, if carried out carefully, can be used quite advantageously by comparison of the particle size with the dimensions of its shadow.

Another method of observation is a modification of an earlier procedure outlined by Marton (*1*), which involved focusing on a dummy specimen and then substituting the true specimen. Von Ardenne (*2*) applied the same principle by providing a shadowing wedge, protecting part of the specimen during focusing. The modification, as used in this laboratory, consists of carrying out the focusing, which requires a beam of relatively high intensity, on a part of the specimen which can be sacrificed. After the best focus is achieved, the beam intensity is reduced to the minimum required for photographic recording and the stage shifted to a part of the specimen which has not been irradiated previously. A micrograph can then be obtained with a minimum of irradiation and corresponding minimum changes. In a variation of the same procedure the whole surface of the specimen is scanned first at very low intensity and the most interesting part preselected. A shift is then made to a less important area which can be sacrificed. This is brought into focus at high intensity and then, after reduction to low intensity, a shift is made back to the good area. Care should be taken that the two areas are far enough removed so that essentially no high-intensity beam can reach the part which has to be protected. In both cases the filament current can be reduced when the image of the filament is produced in the object plane by means of the condenser lens, and increased again to the optimum value when the condenser setting corresponds to the one required for photographic recording.

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Frozen-dried Preparations for the Electron Microscope

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Most native proteins are strongly hydrated whether they are in molecular suspension or form part of an organized biological tissue. When this water is lost during air-drying for electron microscopy, it is likely that the elementary particles of many proteins will shrink and distort. Dehydration cannot now be avoided, and therefore techniques are needed which will desiccate with a minimum of alteration. Quick-freezing and desiccation from the frozen state does this.

Good frozen-dried preparations for electron microscopy can be made easily in the following way: Aluminum strips are substituted for glass microscope slides in mounting the usual collodion- or formvar-covered screens. When ready for use, these metal slides and the screens they carry are placed on a block of metal (approximately $8 \times 3 \times 2$ cm.), precooled with dry ice or liquid air. When a microdrop of solution is applied to, and immediately withdrawn from, screens cooled in this fashion, some will instantly freeze; a tissue such as tendon, touched momentarily to a cold screen, will leave frozen shreds behind. The collodion or formvar substrate can often be omitted when dealing with tissues and with suspensions of elongated particles. The block has sufficient thermal capacity to hold the preparations frozen while they are being made, while both block and preparations are being transferred to a vacuum chamber, and until a high vacuum has been drawn. If the preparations are to be shadow-cast before microscopic examination, vacuum desiccation and shadowing can conveniently take place in the same apparatus without breaking vacuum. The screens on their metal block must remain well below freezing until desiccation has been completed, but by the end of the run they should be warm enough so that moisture from readmitted air does not condense on them. The slow thermal leakage this demands is provided by putting the block on one or more pieces of lightly metal-coated glass.

Electron micrographs have thus far been made of frozen-dried bacteria, of several plant and animal viruses, and of certain tissues. With dilute solutions of the tobacco mosaic and bean mosaic viruses, for example, these pictures are indistinguishable from those of ordinary air-dried preparations; in such instances it would appear that air-drying does not appreciably distort the virus particles. Influenza virus particles have been strikingly full and turgid after freeze-drying. Unusually interesting pictures have been obtained from frozen-dried concentrated solutions of tobacco mosaic protein. In them many rods are associated together in two dimensions to yield areas which look surprisingly like sheets of connective tissue and break up in the same way into fibrous bundles—for example, under impact of the electron beam. Frozen-dried preparations containing appreciable quantities of salt have not yet given useful micrographs, because in its extreme dispersion this salt tends to smear over the fine details that are present.

In this laboratory frozen-dried as well as air-dried preparations are now a routine. Electron micrographs of some of them will shortly be published elsewhere.

Letters to the Editor

On the Mechanism of Action of Folic Acid and Liver Extract in the Treatment of Anemia

In view of the discovery by Spies and co-workers (*S. med. J.*, 1945, 38, 707) that synthetic folic acid has antianemic action in the treatment of human macrocytic anemias, the question of its mechanism of action has become a matter of some interest. Experiments performed in our laboratory (details to be published) may throw considerable light on this problem.

We have produced significant hyperchromic anemias in five normal dogs by the subcutaneous injection of 3 mg. of acetylcholine bromide twice daily for 47 days. Two of these dogs were then treated by the daily injection of liver extract, in addition to acetylcholine. They responded with an increase of reticulocyte percentage and a gradual regeneration of red blood cells to their normal number. Another dog of this series received daily folic acid injections (2 mg.) and responded in a similar manner.

Three dogs were made anemic by the feeding of choline chloride according to the general method reported previously by the author (*Amer. J. Physiol.*, 1944, 142, 402). Two of these dogs were treated with daily injections of folic acid and the third, after serving as an anemic control animal, was treated daily with liver extract. These animals all responded by showing a rise of reticulocytes (to peaks of 3.4–4.2 per cent, from 6 to 9 days after onset of treatment) and a return to normal of their erythrocyte numbers within 20 days, in spite of continued choline feeding.

During anemia, acetylcholine-like activity was detected in extracts of serum of blood drawn from the "choline anemia" dogs at one and one-half hours after the administration of 200 mg. of choline chloride. This activity was markedly diminished after antianemic treatment had been instituted. Cholinesterase activity (determined by an electrometric titration method) of the serum of one dog tested was low during anemia and was increased 12-fold during treatment with liver extract.

Incubation of various dog blood sera with folic acid or liver extract at 37° C. increased their cholinesterase activities by from 0 up to 93 per cent. Similar incubation of one normal human serum with liver or folic acid increased its activity by 15 per cent.