taken on the first few inches (urine spot usually 2″ from top of the strip) and on the portion of the paper beyond the limit of phenol development give the background reading.

The possible application of this method to the testing of the distribution of radioactive sulfur compounds of the urine was tested by rat-feeding experiments in

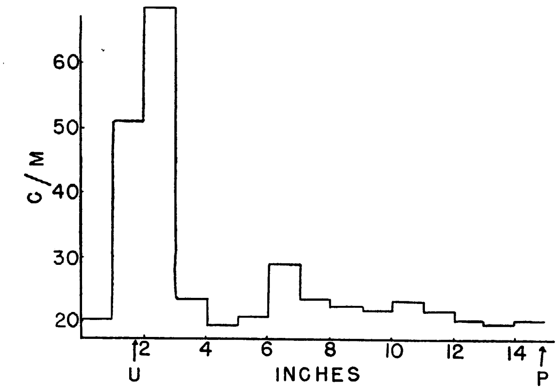

FIG. 2. Radiopapergram of urine from rat fed 5 mg (ca. 350,000 c/m) of radiomethionine: U, urine spot; P, limit of phenol development.

which radioactive methionine was administered alone and with compounds known to influence the urinary sulfur excretion. The radiopapergram of urine from an adult male white rat fed radiomethionine is presented in Fig. 2. The initial peak represents the inorganic sulfate fraction, which, being relatively phenol insoluble, moves slowly down the strip. Treatment of this urine sample with barium chloride prior to development

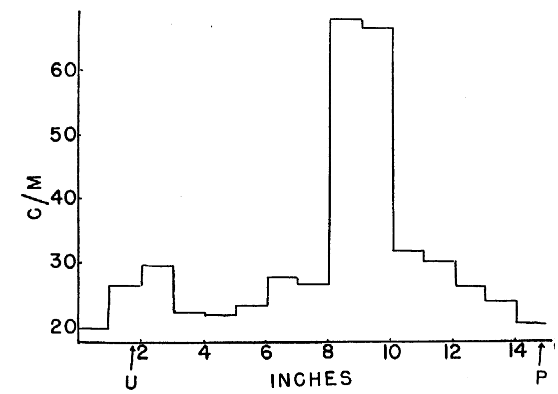

FIG. 3. Radiopapergram of urine from rat fed 5 mg (ca. 350,000 c/m) of radiomethionine followed by a subcutaneous injection of 0.15 ml of benzene: U, urine spot; P, limit of phenol development.

removes this peak. Oral administration of radiomethionine to a rat, followed by an injection of benzene, yielded the urine papergram presented in Fig. 3. The inorganic sulfate peak is not present; the radioactivity peak midway down the strip represents ethereal sulfate, the product of benzene detoxification. Hydrolysis of this urine liberates the phenolic sulfate, and, upon development of hydrolyzed urine on the papergram, the radioactivity is again found in the inorganic sulfate region.

The urine from a rat fed a suspension of bromobenzene in radiomethionine solution yielded a papergram with two radioactivity peaks. The first peak near the urine spot is that of inorganic sulfate; the second peak, at approximately three-quarters of the phenol limit, is due to mercapturic acid, the detoxication product of bromobenzene.

The radiopapergram offers promise as a useful tool in metabolism studies utilizing radioactive isotopes. With the proper refinements and standardizations, the method may be made quantitative. The monotonous chore of reading radioactivity along the paper strip may be eliminated by making the process an automatic one. This may be accomplished by synchronizing a constant speed motor, pulling the paper strip slowly beneath the Geiger counter, with a tape recorder whose pen is motivated by counting impulses coming from the scaler.

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A Simple Attachment to Increase Depth of Focus of Microscope Objectives for Photomicrography

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One of the most important considerations in photomicrography is the attainment of desired definition and perspective. Frequently it becomes necessary in this laboratory to obtain photographs of minute insect specimens of an opaque form, often *in situ*. Such photographs make necessary the use of conventional achromatic microscope objectives having magnifying powers greater than the upper limits of the longer focal lengths of the Micro Tessars. While the achromatic objectives are corrected to the highest degree for their ordinary use, marginal aberration prevents the depth of focus which is often desired. They are designed so as to sacrifice depth of focus in order to obtain the higher resolving power for which they are valued. The apochromatic objectives, because of their finer color correction and increase in usable numerical aperture, are more desirable for ordinary use than the achromatic objectives.

The necessity for *higher magnifications, with some corresponding degree of depth of focus without appreciable loss of definition and resolution,* led to the construction of a simple device by means of which satisfactory photomicrographs of small insect specimens may be made. A device was constructed to reduce sufficiently the

[1] The writer wishes to thank Dr. Laurence E. Dodd, in charge of Geometric Optics in the Physics Department of this University, for his review and criticism of this paper.

marginal aberration of the field encountered when using standard microscope objectives. This device serves the purpose of an iris diaphragm in providing a means of adjusting an otherwise permanent aperture. Reduction in aperture is effected by moving a thin metallic ribbon, perforated with holes of various sizes, across the field of vision directly under, and in gentle contact with, the microscope objective. When mounted on a mechanical stage and moved from side to side, so that the holes increase or decrease with the movement, the effect is comparable to the opening and closing of an iris diaphragm.

When the mechanical stage is used to hold the perforated strip in place, the specimen to be photographed should be directly beneath. This enables the substage condenser to be utilized to advantage. With the top element of the condenser removed, the exposed threaded ring provides a secure base upon which a small plate of ground glass may be placed. It has been found that grinding both surfaces of the plate serves to diffuse adequately strong condensed light, when transmitted central illumination becomes necessary. Diffused light may be made intense without the distracting marginal glare often encountered when the light is directly transmitted without diffusion. A small cork with the center bored out, and with a microscope-slide coverslip glued over one end, provides a satisfactory pedestal. The specimen may then be placed on the coverslip surface to receive the full benefits of the transmitted diffused lighting.

When using the substage condenser for holding the specimen, one can move vertically the entire assembly holding the specimens both above and below the surface of the microscope stage. This makes possible the manipulation of the mechanical stage holding the perforated metal strip without any interference with the specimen. Once the metal strip is locked into position, with its largest hole immediately below the objective, the latter is then carefully lowered until contact is made. The substage condenser, holding the object to be photographed, may then be elevated into approximate focus; the final focusing is completed with the fine adjustment. It then becomes a simple matter to move the device, thus bringing progressively smaller holes beneath the microscope objective until the required depth of focus is obtained.

It must be borne in mind that when extreme depth of focus is attained, a certain amount of resolving power is necessarily sacrificed. The mechanical stage should be moved into position in order to utilize the smallest aperture in the perforated metal strip consistent with adequate definition. This may not necessarily be the smallest aperture in the strip.

As the device was constructed for use in this laboratory, aluminum foil with a gauge of 0.009″ was utilized. Designed to fit the mechanical stage, the over-all length of the formed aluminum strip is 3″, with an arbitrary width of ⅜″, in order to provide sufficient clearance over the object to be photographed, yet not in excess of the working distance of a 10X microscope objective.

The formed strip may be fixed to the mechanical stage or mounted on a plastic "slide" held by the mechanical stage, but with its center cut out to allow clearance for the photographed object (see Fig. 1). The holes drilled in the strip were of the following drill sizes: #'s 25, 31, 37, 41, 45, 49, 53, 57, and 60. Particular care was taken in burring the holes to prevent scratching of the microscope objective. Drilling the holes in a straight line and close to the outer edge of the strip prevents interference with frontal lighting and also provides ease of operation when selecting the proper size of aperture.

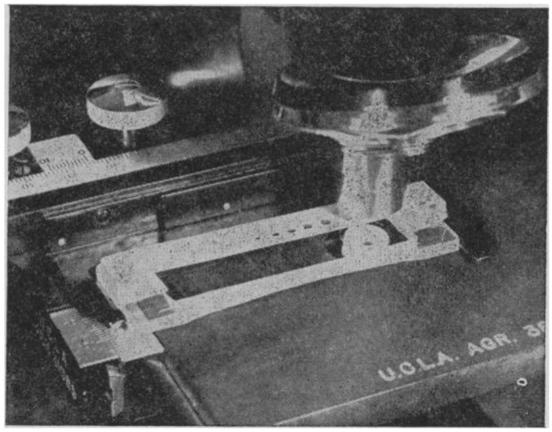

FIG. 1. Microscope attachment used to increase depth of focus in photomicrography.

With a little practice under visual observation, one soon becomes familiar with the use of this attachment. Manipulation with various heights of the substage-condenser pedestal and experimentation with various angles of lighting soon lead to easy and rapid use of the device.

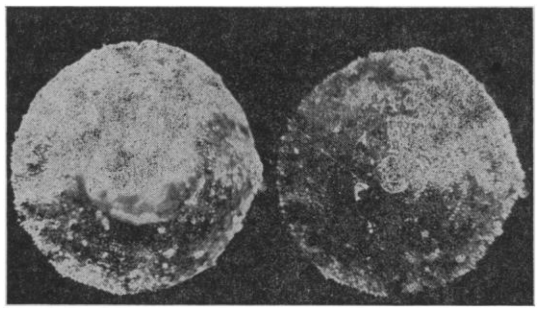

FIG. 2. California red scale photographed with top oblique lighting: A (*left*), without aid of microscope attachment to increase depth of focus; B (*right*), same specimen photographed with the aid of the attachment.

Fig. 2 shows a photomicrograph of the dorsal aspect of the California red scale, *Aonidella aurantii* (Mask.). The lighting originates simultaneously from above and from an oblique source. The optical equipment used was a 10X achromatic objective and a 5X Huygenian ocular. Fig. 2A shows the result of a compromised focal adjustment made to embrace as much of the specimen as the scope of the optical equipment would allow, while Fig. 2B shows the same specimen, using identical lighting and equipment as in A, but using the microscope attachment to increase the depth of focus.