

larvae. Apparently the products of fermentation are injurious to them.

As a routine part of the rearing of blowflies we now add shavings or sawdust when the larvae reach the crawling prepupal stage, and, when the first pupae appear, the whole mass is poured from the rearing jar into a shallow enameled pan, where it dries out as pupation proceeds. The pupae are then either left in the dry material for emergence or sorted out. As in the case of houseflies, the latter results in a somewhat better emergence.

With regard to the sawdust or shavings used in the methods described, the best material we have found to date is the very coarse sawdust produced in the sawing of fresh timber with a large circular saw. Fine shavings from a wood-working shop are satisfactory, however. Ordinary fine sawdust packs too tightly, and coarse shavings are too loose. Preliminary work with cellucotton, such as is used for packaging, indicates that this material may also have value. Obviously, the moisture content of the shavings or sawdust is an important factor in their use.

In the previous paper (2), the question of whether blowflies reared on meat will oviposit on dog biscuit was raised. This can now be answered in the affirmative. We have obtained oviposition on moistened dog biscuit by *Phormia regina* and by other species of blowflies captured wild.

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## Use of Papergrams in the Study of the Urinary Excretion of Radioactive Sulfur Compounds<sup>1</sup>

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In applying the paper partition chromatography technique (1) to the detection and separation of constituents of biological fluids, Dent (2, 3) has developed a method that is convenient and particularly suitable to the study of small-volume samples. The present pub-

<sup>1</sup>These investigations were conducted under the auspices of the Josiah Macy, Jr. Foundation, Conference on Liver Injury, and Subcommittee on Tagged Methionine, with the participation of the Schools of Medicine of the Universities of Wisconsin, California, Pennsylvania, and Emory University.

The term "papergram" is more applicable than paper chromatogram, since color is not the guide to the distribution of the components being separated.

lication deals with the application of this technique to the study of the distribution of radioactive sulfur compounds excreted in the urine of rats fed methionine containing S<sup>35</sup>.

One-dimensional papergrams were prepared in the manner described by Dent (3), care being taken to confine the urine spot to as small an area as possible. A total of 0.06 ml of urine may be deposited on the filter paper within a circular area 1 cm in diameter if applied in 0.01-ml aliquots and allowed to dry before the next application. The papergrams are developed overnight

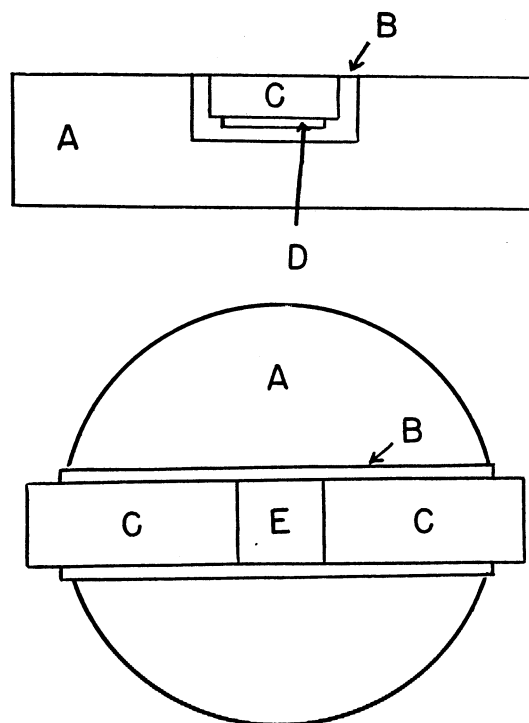


FIG. 1. Slotted base for reading of the papergram: A, lead base; B, machined brass part; C, sliding bar; D, slot; E, aperture.

The papergram is passed into the slot, D, until the first inch is under the square aperture, E, thereby exposing this section of the paper to the Geiger tube. A radioactivity count is taken, and the paper strip is then pulled into position for the reading of the next and succeeding areas.

with liquefied phenol, the phenol traveling down 11-14" from the top of the paper. After removal of the phenol by heating, the paper is treated with some chromogenic reagent (ninhydrin) to outline the pathway followed by the urinary constituents. A strip, 1" wide, enclosing all the colored areas and presumably all the radioactivity, is then cut from the larger piece of filter paper. The distribution of the radioactivity along the strip is determined by means of a Geiger counter, mounted on a shielded base, so constructed that the papergram may be passed beneath the window of the counter. The base is shown in Fig. 1. The radioactivity readings

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

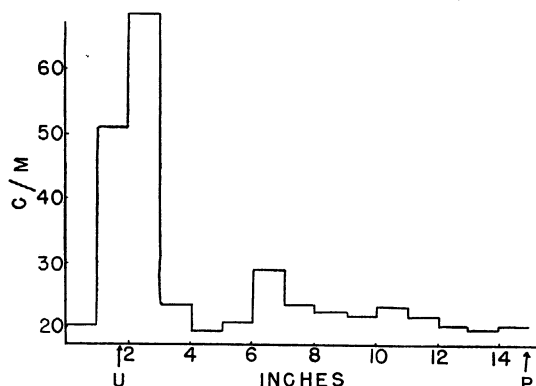


FIG. 2. Radiopapergram of urine from rat fed 5 mg (ca. 350,000 c/m) of radiomethionine: U, urine spot; P, limit of phenol development.

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

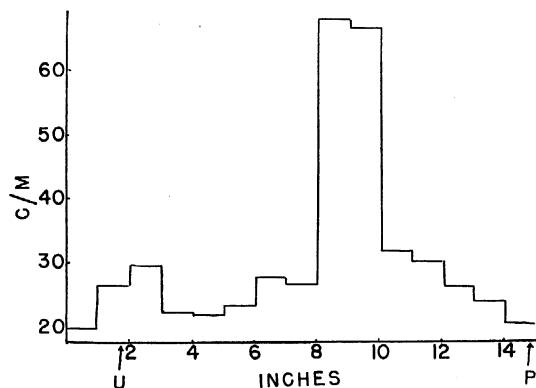


FIG. 3. Radiopapergram of urine from rat fed 5 mg (ca. 350,000 c/m) of radiomethionine followed by a subcutaneous injection of 0.15 ml of benzene: U, urine spot; P, limit of phenol development.

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.

#### References

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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

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