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.

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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

followed closely so as to add fresh droplets as the xylol and chloralphenol evaporate. Finally the material is mounted in pure balsam and can be kept indefinitely.

Counterindication of this technique holds only for hyaline spores or mycelia which are scarcely visible in balsam. But wherever a slight coloration or a sufficiently thick membrane is present, details are sufficiently visible to permit taxonomical or anatomical observations.

There is another drawback to this technique when free spores are present on the slide. These can be displaced or drawn out from under the cover slip when the slide is levigated with the two media. When such is foreseen, one can place the material directly in a mixture of chloralphenol and balsam diluted in xylol in the proportion of two droplets to one. The chloralphenol insures the clarification of the material and the penetration of the balsam. With the cover slip on, the slide is gently heated until the chloralphenol has completely evaporated. Here again, attention must be paid to feeding fresh droplets of Canada balsam as the mounting medium dries up.

This latter procedure does not apply to fresh material but only to herbarium material or one that has been dried beforehand.

This technique has been very useful to the writer to prepare quickly permanent mounts of abundant herbarium material that had to be examined at short notice, while keeping a permanent record of the observations.

Chloralphenol is prepared by mixing by weight two parts of chloral hydrate to one part of phenol crystals. The mixture, liquefied by gently heating it on a flame, can be kept in dropping bottles.

This technique may not be totally unknown, as Langeron seems to infer. In any case, the writer has seen no reference in the botanical or phytopathological literature to its having been used.

Fluorophotometric Determination of Rutin and Other Flavones

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A great deal of interest has developed in the therapeutic use of rutin for the treatment of reduced capillary resistance in certain hemorrhagic diseases (*1*, *3*). Rutin is a flavone glucoside which yields quercetin, glucose, and rhamnose on hydrolysis with dilute acids (*2*). Most flavones react with boric acid to form highly colored derivatives which can be adapted to colorimetric determinations (*5*, *6*). We have applied the boric acid reaction to the determination of rutin and have also improved the sensitivity of the method by measuring the intensity of fluorescence with a photometer, instead of measuring the optical density of the colored solution. We have also found that the fluorometric method can be used to advantage with purified quercetin, and presumably with other flavones which react with boric acid.

A stock solution of recrystallized rutin[1] in dry acetone was diluted to give a series of standards containing known concentrations of rutin. The boric acid reagent was prepared by mixing equal volumes of a saturated solution of boric acid in acetone and a 10 per cent citric acid solution in acetone, as described by Wilson, Weatherby, and Bock (*6*). The reagent was added to the rutin standards in the ratio of four volumes to one of rutin solution. Colorimetric measurements were made with a Klett-Summerson photoelectric colorimeter using a blue filter (No. 42), and the fluorescence measurements were made with a Pfaltz and Bauer Model B fluorophotometer. The mercury vapor light in the fluorophotometer was passed through a set of filters having a peak transmission in the violet band at 430–440 mμ. The degree of fluorescence was measured with a photocell through a yellow filter having a sharp cutoff at 520 mμ. Fluorescence was also observed using ultraviolet light below 370 mμ; but the photometer readings were higher with the violet light source which was finally chosen for this work. The rutin-boric acid solutions exhibited a green fluorescence which, on examination with a spectroscope, appeared to extend from 510 mμ to 620 mμ.

Calibration of the fluorophotometer with rutin standards produced the results shown in Table 1. The degree of fluorescence appears to be directly related to the concentration of rutin and affords a convenient method for its determination. The advantages of fluorimetry over colorimetry are also demonstrated in Table 1, where the readings with different concentrations of rutin are compared. However, the boro-citric reagent itself exhibits some fluorescence which varies with different lots of reagent (*6*). The effects of moisture, concentration of reagents, time of standing, and other variables have been worked out, and the fluorimetric method is now being applied to the determination of rutin in biological materials, using procedures for extraction similar to those already developed for flavones (*4*, *6*).

TABLE 1

COMPARISON OF FLUORIMETRIC AND COLORIMETRIC READINGS WITH RUTIN-BORIC ACID SOLUTIONS

Rutin/ml. sample (μg.)	Colorimeter readings	Fluorimeter readings
10.0	27.0	67.0
8.0	22.0	55.0
6.0	17.0	43.0
4.0	12.0	30.0
2.0	8.0	16.0
1.0	5.0	9.0
0.50	3.5	5.5
0.25	2.0	4.0
0.10	1.0	3.0
0	0	2.0

[1] The rutin used in these experiments was obtained through the courtesy of Dr. M. J. Copley, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia.

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