thin in the Kline test and both the Kolmer and Debains complement fixation tests.

We have also prepared antigens with D-α-dimyristoyl lecithin and DL-α-dimyristoyl lecithin and have used them in the V.D.R.L. slide test in parallel with L-α-dimyristoyl lecithin antigen of the same composition. Very similar results were obtained. There was also good agreement when the 3 lecithins were used in parallel in the Kolmer test.

Stearoyl glycollecithin was used as a substitute for lecithin in a wide range of concentrations in the V.D.R.L. slide test. With syphilitic sera, flocculation occurred but the various antigens were undersensitive compared with V.D.R.L. slide test antigen. Coarse particles, and often small aggregates of particles, were observed with normal sera. In the Kolmer test a level of sensitivity approximately that of Kolmer antigen was obtained with the following antigen mixture: cardiolipin, 0.03%; glycollecithin, 0.075%; cholesterol, 0.3%. No anticomplementary or hemolytic properties were detected.

Difficulty was encountered in the substitution of L-α-dimyristoyl cephalin for beef-heart lecithin due to the limited solubility of the material in absolute ethyl alcohol. Two antigen mixtures were prepared for use in the Kolmer test and they proved to be anticomplementary.

No serological reactivity was observed when dipalmitoyl-L-α-glycerophosphoric acid monocholine salt was used in place of natural lecithin in V.D.R.L. slide test antigen.

The phosphatidic acid, tetramyristoyl-bis-(L-α-glyceryl) phosphoric acid was substituted for cardiolipin in antigen mixtures prepared for the V.D.R.L. test. Flocculation occurred with strongly positive syphilitic sera. When normal sera were examined, the test mixtures were often coarse and difficult to read. In the Kolmer test, certain phosphatidic acid antigen mixtures showed definite reactivity with positive sera, but even the most reactive (phosphatidic acid, 0.08%; lecithin, 0.033%; cholesterol, 0.3%) was less sensitive than Kolmer lipoidal antigen. Possibly other phosphatidic acids may prove to be more satisfactory. Faure (*7*) has found that unsaturated phosphatidic acids extracted from plants can be substituted for cardiolipin but that the resultant antigen is less reactive.

The use of pure synthetic compounds in the preparation of antigens would offer certain advantages in the standardization of serodiagnostic tests for syphilis. In addition, the investigation holds some promise of revealing the mechanism of reaction and perhaps the molecular groupings which are involved. Further studies are being conducted.

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Comments and Communications

Chromatogram Spotting Apparatus[1,2]

THE chromatogram spotting apparatus[3] shown was designed and built in an attempt to standardize the technique of placing liquid material on paper chromatograms in definite amounts. Experience in this laboratory has shown that considerable variability in chromatograms results when several individuals are doing the spotting, as occurs in the case of a paper chromatographic study involving samples of many field replications. This equipment enables the worker to standardize the technique and facilitate ease of operation, thus making it possible for less experienced employees to do uniform, high quality spotting.

The principal error in spotting seems to be due to a failure to place the micropipette at right angles to the paper. This often results in an irregular spot. The parallelogram arm pipette holder eliminates this error. A T-square notched at regular intervals reduces the usual time-consuming process of measuring and marking one-dimensional papers to be spotted. The lighted recess or well in the board directly under the spotting

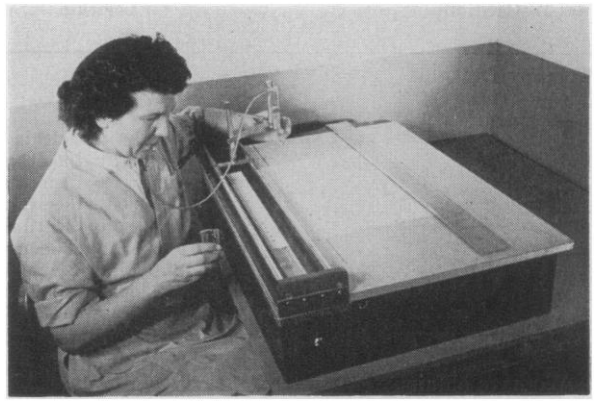

FIG. 1.

[1] Published with approval of the director, Colorado Agricultural Experiment Station, as G. S. paper No. 525.

[2] This equipment was developed during the course of a biochemical investigation supported in part by the Herman Frasch Foundation.

[3] A complete set of detailed blueprints and photographs may be obtained for $5.00 to cover cost of preparation by writing directly to Clark Livingston, Chemistry Department, Colorado A. & M. College, Fort Collins, Colorado.

area relieves eye strain and provides a warm air space below the paper, thereby increasing the rate at which the spots will dry. A small pull-type drawer serves as a handy storage place for pipettes, etc. The level of the table on which the apparatus is placed is adjusted to bring the level of the pipette to shoulder-height when the operator is in a sitting position.

CLARK H. LIVINGSTON
MERLE G. PAYNE
JESS L. FULTS
ROGER M. BLOUCH
Departments of Chemistry and Botany and Plant Pathology
Colorado Agricultural Experiment Station
Fort Collins, Colorado

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Zoological Collecting Expeditions and the Salvage of Animal Bloods for Comparative Serology

ADEQUATE collections of individuals and species of animals are indispensable in the present-day approach to the problems of animal systematics. This requires that much collecting remains for the future and that even some of the territories previously surveyed may need to be revisited. We call to the attention of those planning and participating in zoological collecting expeditions the possibility and the desirability of saving the blood and obtaining the sera of representative animals in order that a thorough study of the amounts of serological and biochemical correspondence of their blood proteins may be made.

Useful samples of animal bloods and sera may be obtained even with the barest minimum of equipment and facilities. Such samples may serve in "first approximation" studies and may help to indicate where more careful testing is needed.

Let us be realistic and assume that most zoological collecting expeditions will be: (1) short-handed and incapable of devoting more than a minimum of time to extra duties, however desirable from other points of view; (2) unequipped for refined serological collecting; and (3) inexperienced in the standard procedures for collecting and handling animal bloods. And let us also be optimistic in assuming that some members of such expeditions would be willing to give limited help to us and save the bloods and sera of some of the animals obtained in normal routine collecting. What could such people do that would require the very minimum of time, experience, and equipment?

We will discuss the possibilities for collecting animal bloods on two levels, viz., (1) that where no facilities for collecting fluid blood are available and (2) that where fluid blood and sera may be collected but at the minimum level of equipment.

1. Collection of blood without facilities for handling volumes of fluid blood or sera: The simple procedure, for small birds or mammals, is to make use of filter paper or towelling paper to soak up the blood from wounds or the blood and sera from the animal's carcass as it is being skinned. Care should be taken to keep the paper free of fat. Such blood and sera squeezed from the carcasses may be supplemented by blood obtained directly upon cutting open the heart and major vessels. It would be desirable to obtain the equivalent of a 2-inch square of soaked filter paper from a single small bird or mammal, but often this is not possible. In such cases the procedure may be repeated for several individuals of the same species—all on one piece of filter paper if the identification is certain. For a very rare small specimen we will be glad to get anything at all. The fact is that sometimes it is possible to identify the species from a spot of blood no larger than a matchhead. For animals as large as a red fox or larger, it should be possible to obtain the equivalent of a square foot or more of soaked paper from one or more individuals.

The soaked paper should be hung up in the shade to dry at prevailing temperatures, carefully shielded from other papers and from visitations by insects. When thoroughly dry, complete the labelling by writing in pencil, directly on the paper, the scientific name of the specimen, date, locality, whether sample is pooled or single, and name of the collector. If the identification is uncertain and the skin or other parts of the specimen are being preserved for later identification, indicate the specimen number and institution. Wrap the filter paper in protective paper and keep dry until sent to the Serological Museum.

2. Blood collecting where containers for fluid blood, bottles for serum, and preservatives are available: Any clean jar or container may be used for collecting fluid blood from fresh wounds or cuts. Allow the blood to clot in as cool a place as possible (but whole blood should not be frozen). After it has clotted, loosen the blood from the sides of the container and allow it to stand for several hours at room temperatures or overnight at refrigerator temperatures. During this time the serum will usually express itself from the clot as the clot shrinks. Pour off the serum as clear as possible into a serum bottle and add the preservative. A standard preservative is 2% formalin prepared by adding 2 ml of commercial formalin (equivalent to about 40% formaldehyde) to about 98 ml of fresh water. Use one part of this standard 2% formalin to nine parts of serum. The final concentration of formalin in the serum is thus 0.2%, and this has served as a very good field preservative.

If no suitable bottles are available for the storage of the serum, it may be soaked up directly onto the filter paper, and dried as for whole blood.

It is our hope that some useful samples for serological study may be obtained in these ways and that bloods which would otherwise have been lost may thus be salvaged. It is our hope also that through the modest but considerate help of *many* individuals and expeditions it will not be necessary to organize special collecting expeditions to obtain the needed sera. We would appreciate very much therefore, being in-