cross in the second microscope when they would have been added or *vice versa*. Since the cross lies farther to the left and lower down than any point within the cover glass, any incorrect value for the final coordinates (x″, y″) on account of the scales running in opposite directions will give a point entirely outside the cover glass. Hence the mistake will be immediately evident and easily remedied.

The accuracy of the method depends upon the precision with which the mechanical stages are made and graduated and upon the care with which the coordinates are read and recorded. Except for a few petrographic stages graduated to 0.01 mm, standard mechanical stages, either "built in" or attachable, are graduated to 0.1 mm and can be readily estimated to 0.05 mm. This means that an object can be located within a square 0.05 mm on a side, which, considering the fact that the field of view of a 1.8 mm objective and 10x ocular is 0.2 mm in diameter, is satisfactory even for a very small object. It would help considerably if all microscope manufacturers adopted a uniform system in the graduation of mechanical stages. The two largest manufacturers of microscopes in this country follow the same system for the horizontal movements of their stages, but have the numbers increasing in opposite directions on the vertical movements. The best method would be to have the stages graduated so as to read 0–80 mm from left to right on the horizontal movement and 80–130 from bottom to top on the vertical movement. This would place all the coordinates in the first quadrant, giving positive values. Any other arrangement introduces negative numbers for at least one of the coordinates.

This method has been used by the writer over a period of years and found to be entirely satisfactory. Objects located eleven years ago can be found quickly to-day. Furthermore, the slides, with the necessary data, may be sent to any one having a graduated mechanical stage on his microscope with the assurance that he will find the objects easily by the application of the above method.

K. E. LOHMAN

U. S. GEOLOGICAL SURVEY

AVOIDANCE OF EMULSIFICATION IN DEFATTING OPERATIONS

IN previous work on the fatty oil of digitalis seed[1] difficulty had been experienced in the separation of the fatty oil from the alcoholic concentrate because of the permanence of emulsion formed in shaking the hydroalcoholic extract with petroleum ether. Hence, it was suggested to one of us to try an adaptation of the method long used to remove alkaloids by means of ether or chloroform from solutions with which these alkaloidal solvents are immiscible. Reference is had to the method of allowing these solvents to bubble through the liquid from which the alkaloid is to be removed. The large quantity of concentrated tincture seemed to render the conventional long narrow tube impracticable; hence, a percolator was substituted therefor. To the surprise of the operator, the petroleum ether, when passed into the bottom of the percolator, did not bubble through the hydro-alcoholic fatty extract, but the bubbles quickly blended with the extract it was intended to defat. The result was a solution, though not clear at first. Upon the further addition of petroleum ether a sort of emulsoid resulted. Another surprise was in store when, after the continued addition of petroleum ether, the apparent diphase separated into two layers, the clear petroleum ether solution rising to the top without a trace of emulsification. The addition of petroleum ether which, after the breaking up of the emulsoid, bubbled through the hydro-alcoholic layer of extract was continued until the latter was completely defatted.

In order to ascertain whether this technique is applicable to a wider range of plant products, it was applied to the defatting of a concentrated alcoholic extract of linseed with a high fatty oil content. It was also applied to the removal of the non-saponifiable matter with ether from the aqueous soap solution of milkweed oil. In both instances it proved equally successful. Thinking that the method may prove useful to others who have been annoyed by obstinate emulsions in the defatting of hydro-alcoholic extracts, it is herewith described to be tried out if they see fit.

Fig. 1 shows the set-up before it is placed in operation. Aspirator bottle, A, is filled with petroleum ether. Percolator, P, contains the hydro-alcoholic extract to be defatted, introduced from separatory funnel, D; the amount of hydro-alcoholic extract that can advantageously be defatted in one operation varies with the fat content. It may be best not to fill the percolator more than one third. Screw cock S controls the flow of petroleum ether. Screw cock S_1 enables the draining of the percolator after defatting has taken place. Flask F is used to collect any excess petroleum ether containing fat removed from the alcoholic extract. The washed hydro-alcoholic extract is drained from the bottom of the percolator into flask F_1. After its removal the upper layer of petroleum ether containing dissolved fatty oil can likewise be removed by this means. Separatory funnel D is used to recharge the percolator with fresh hydro-alcoholic concentrate. Tube, T, functions as a syphon removing any excess of fat-containing petroleum ether. It is necessary that cork C fit as tightly as possible; hence it should, if necessary, be sealed with a suitable agent.

As already pointed out, it may prove advantageous not to fill the percolator more than one third. When

[1] S. H. Culter, *Am. Jour. Pharm.*, 102: 545, 1930.

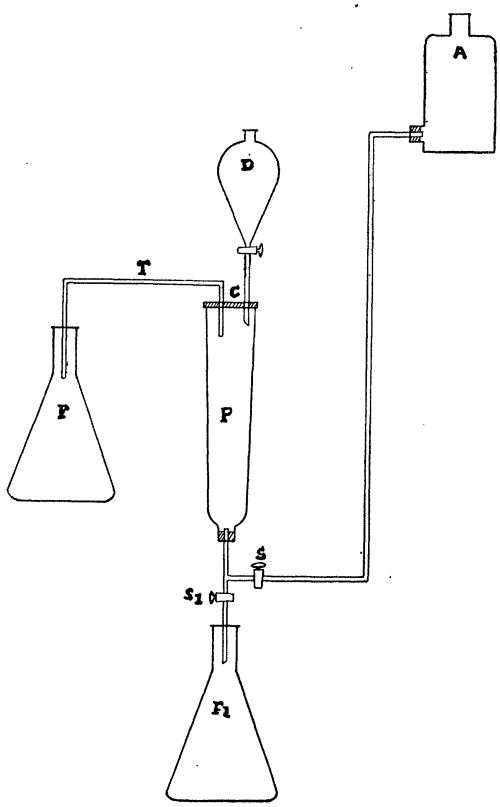

FIG. 1.

the petroleum ether flow was started, it produced a clouding effect at the point of entrance. However, this disappeared almost immediately. Upon further addition of the petroleum ether, the clouding increased gradually until the whole solution was uniformly cloudy, while the volume had increased materially. Evidently, this is the emulsification stage. As more petroleum ether was added to the solution or emulsion, a petroleum ether layer containing fat began to separate above. As still more immiscible solvent was added, the size of the petroleum ether layer increased, whereas that of the lower hydro-alcoholic layer diminished. At the same time the syphon began to function due to the air chamber formed in the top of the percolator. In this manner the excess of the petroleum ether layer was forced out. The bubbling up of petroleum ether through the lower hydro-alcoholic layer was continued until all the fatty oil had been removed. This point can be judged roughly when the petroleum ether that separates is no longer colored. Shortly after the separation of the petroleum ether layer, the lower hydro-alcoholic layer became clear. Upon the addition of more petroleum ether, the original hydro-alcoholic solution receded to a volume slightly below the volume introduced at first.

Continued extraction caused some of the material in the hydro-alcoholic extract, other than fat, to separate out on the walls of the percolator. Hence this method not only served as a means of avoiding emulsification, but also brought about a separation of a third substance, at least in this instance.

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SPECIAL ARTICLES

RELATIONSHIP OF THE VIRUSES OF VESICULAR STOMATITIS AND OF EQUINE ENCEPHALOMYELITIS

THE virus of equine encephalomyelitis, recovered by Meyer, Haring, and Howitt[1] from affected horses, is regarded by them to be distinct from the incitant of botulism, "forage poisoning," Borna's disease, poliomyelitis, and apparently, from that of enzootic encephalitis of the Moussu-Marchand type.[1,2] In view of the fact that the horse is the natural host for this disease and also for vesicular stomatitis, we undertook a comparison of the properties of the viruses obtained from both infections. We wish to thank Miss B. Howitt, of the George Williams Hooper Foundation of the University of California, for a specimen of the encephalomyelitis virus.

The following series of comparative tests were made:

Intracerebral Inoculation:[3] The intracerebral inoculation of guinea-pigs, white mice, *Macacus rhesus* and *cynomolgus* monkeys with either virus induces in each instance fatal encephalomyelitis,[4] characterized usually by the same period of incubation and set of symptoms. The rabbit, however, is much more resistant to the two viruses than the other animals mentioned, and 24 to 48 hour old chicks are unaffected by them. The viruses can be recovered from the submaxillary and parotid glands, blood, brain, spinal fluid, lung, spleen, liver and kidney of monkeys, guinea-pigs, and mice experimentally inoculated with either one. The gross and microscopic changes in the brain

[1] K. F. Meyer, C. M. Haring and B. Howitt, SCIENCE, 74: 227, 1931; *Jour. Am. Vet. Med. Assn.*, 79 (n. s. 32): 376, 1931.

[2] B. Howitt, *Proc. Soc. Exp. Biol. and Med.*, 29: 118, 1931; K. F. Meyer, *Ann. Int. Med.*, 6: 645, 1932.

[3] All operations were performed under ether anesthesia.

[4] For a description of experimental vesicular stomatitis pathology, see H. R. Cox and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 30: 653 and 654, 1933.