one hour after decerebration is the average of .11, .09, .14; the chronaxie of .12 for central stimulation is the average of .12, .12, .14; 2 hours after decerebration the value .09 is the average of .08, .08, .10 for peripheral stimulation and .08 is the average of .09, .06, .08 for central stimulation.

The chronaxie of control rats, i. e., non-morphinized, on high calcium diet and "parathormone" differed but little from those on low calcium diet. This is in contrast to the immediate effects of intravenous injection of a soluble calcium salt into the decerebrate cat, where the rheobase is raised and the chronaxie lowered.[2] On the last day of addiction both the "central" and "peripheral" chronaxies of rats on the high calcium diet and "parathormone" were slightly, but not significantly, lower than those of rats on the low calcium diet. During the 3 day period of withdrawal from morphine, the chronaxies were, if anything, slightly lower in practically every case, the differences again being hardly significant.

It may be said, therefore, that neither in normal rats nor in those subjected to morphine addiction and withdrawal does a diet rich in calcium and injection of "parathormone" significantly change the chronaxie of a typical motor nerve or of the flexion reflex.

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Standardization and Relative Purification Technique with Plant Virus Preparations.*

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The usual procedure in the preparation of an infective juice for inoculation or other experimental purposes, in the case of a virus like that of typical tobacco mosaic, consists first in thoroughly crushing or grinding the fresh tissue and then separating the coarser material by filtering off the clearer juice through cotton or cloth. Pressure also may be applied to the crude pulp. The crude juice thus prepared will contain the virus in what appears to be the original concentration. Often this procedure may be improved

[2] Woden, L.-J., *C. R. Soc. Biol.*, Paris, 1931, **106**, 462.

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upon by a simple freezing and pressure technique. With the latter the softer tissues (young stems and leaves) are frozen for a few hours at —20°C. or below, for a period of 4 hours or less, then pressure of 10,000 lbs. or more per square inch is applied. With this treatment a clearer juice is obtainable.

By the method of grinding commonly employed, the crude juice contains varying quantities of starch, proteins and other solid and highly viscous reserve products; likewise chlorophyll, plastid, and protoplasmic particles. If ground in a mortar there is also a large fraction of comminuted cell wall material. The presence of these coarser suspension and colloidal particles is unfortunate in such studies as involve, for example, filtration, adsorption and aggregation, cataphoresis, or various other physical or chemical treatments. Moreover, these coarse particles may vary widely in quantity, and will be related to significant changes in viscosity with plants grown under different environmental conditions. They also interfere with quantitative work involving accurate standardization.

The elimination of coarser particles has been variously accomplished, filtration and centrifugation being often employed. The ordinary laboratory centrifuge alone is inadequate. Filtration through porous filter candles or even alundum cups is slow and likely to give a product very diverse in virus concentration, depending upon kind and quantity of viscous materials present.

In the development of a tentative standard method I have had in mind not a purified virus,[1] but rather a virus suspension reasonably free of coarser materials and still in a reasonably "natural" environment of other solutes. Various clarification processes have been tried. The method finally employed involves removal by precipitation and perhaps selective adsorption of the major part of the contaminating particles by means of diatomaceous earth, leaving the virus in suspension in the clarified juice. The finer grades of commercial diatomaceous earth (Kieselguhr) may be employed, but I have used for ordinary work a special product known as supercelite. Diatomaceous earth is widely used in various filtration processes.

A simple, standard procedure is as follows: The crude juice

[1] Various studies have been carried out by other investigators looking toward the purification and standardization of plant viruses, among which are the following: Brewer, P. H., Kraybill, H. R., Samson, R. W., and Gardner, M. W., *Phytopath.*, 1930, **20**, 943; McKinney, H. H., *J. Agric. Res.*, 1927, **35**, 13; Vinson, C. G., *Phytopath.*, 1932, **22**, 965; Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14; Vinson, C. G., and Petre, A. W., *Contrib. Boyce Thompson Inst.*, 1931, **3**, 131.

from diseased plants, (infectious juice, designated hereafter I.J) is obtained by grinding finely the leaves and young stems in a food mill and then expressing the juice through a double thickness of cheese cloth. This full-strength juice (which may be written 1/1) may be diluted, for best results, with 9 parts of distilled water (= 1/10 I.J.); to this is added with stirring 10 gm. of "celite" per 100 cc. of the diluted I.J. This is allowed to stand 30 minutes, shaking frequently, the preparation is then centrifuged at about 4000 r.p.m. for 4-5 minutes. The resulting supernatant liquid is, or should be, clear and very slightly flavous in color. All material so treated will be briefly referred to as C.T.I.J. (celite treated infectious juice). If the natural (1/1) I.J. is employed, the removal of larger particles and pigment is not so complete; with 1/5 I.J. the purity is intermediate, but obviously this last may be a desirable concentration for the treatment when it is desired to inoculate with 1/10 I.J., and when subsequent treatment of the virus may necessitate further dilution with a liquid.

The 1/10 C.T.I.J. may be prepared as clear and free from color as many of the more elaborately "purified" virus preparations, if the operator selects the top parts only (ca. 10 cm. of the leafy shoot) from diseased plants growing rapidly under favorable conditions. Color of the sample then seems to be related especially to yellowing of the plants, low humidity, and other unsatisfactory conditions.

All satisfactory methods of establishing a known titer of "artificially" communicable plant viruses must consider, (1) dilution, and (2) technique of inoculation. While water (especially distilled water) is commonly used in dilution, it is not certain that this procedure is best. This paper will not deal quantitatively with this point, but an adequate dilution is necessary to arrive at a satisfactory estimate of influences and reagents upon the virus. With the needle-prick and scratch technique of inoculation here used a dilution of 1/1000 is about the highest with which a disease percentage of 90-100 may be rather consistently obtained. Other factors, however, influence the disease percentage.

This proximate purification by means of diatomaceous earth adds nothing undesirable to the virus suspension, does not noticeably shift the pH, and yields a product which may be further used in purification work. Greater dilutions (1/100, 1/1000, etc.) may be prepared in the usual way, with distilled water, or with juice prepared from healthy plants.

If it is very desirable to decolorize the virus suspension still further, also undoubtedly removing additional proteins, this may be accomplished by filtering through a thin stratum of charcoal on an asbestos mat in a Gooch crucible, or shaking up momentarily with certain grades of charcoal.

Various charcoals differ widely in the capacity to "clarify" any particular liquid. Norite, Nuchar 2, XXX, W, 00, give rather rapid decolorization of the tobacco virus suspension, but Norite and Nuchar XXX especially are among the charcoals which strongly, and more or less irreversibly, adsorb or inactivate the tobacco virus from tobacco, so that the use of various charcoals is to be recommended only in special cases when decolorization is important, and when also adequate controls may be provided. It seems probable that a special study would need to be made of the relation of any charcoal to the particular virus juice complex.

The influence of the coarser and more viscous particles of the crude juice on filtration of the virus requires special consideration. Comparing a sample of 1/10 C.T.I.J. with the same concentration of natural I.J., the former, the treated juice, had the following advantages: (a) rapidity of filtration, (b) filtration at lower pressure, (c) relative constancy of virus concentration in the filtrate, and (d) higher virus concentration of the filtrate. For example, in one experiment in which a 12 cm. Mandler filter candle was used with the usual mantle and agar set-up, the filter flask being connected with a water pump aspirator, the celite-treated I.J. filtered readily and the air pressure was reduced to about half an atmosphere by the time the desired amount of filtrate, 15 cc., was obtained. With the natural 1/10 I.J. the pressure was reduced to the full capacity of the apparatus, about one-fifteenth of an atmosphere, and a longer time interval was required to obtain 15 cc. These two samples were tested for efficiency in disease production, with results as shown in Table 1.

It is clear that the viscous materials in the natural juice markedly impeded the passage of the virus particles across the walls of the filter. The infectiousness of the filtered celite-treated juice is not far below, and usually more nearly the same as, the unfiltered controls (averaging 90-100% diseased at a dilution of 1/1000).

The results of numerous experiments have shown clearly that the celite treatment has no marked influence, either immediate or delayed, on the activity of the virus of typical tobacco mosaic. (Table 2).

TABLE I.

Infectiousness of virus of typical tobacco mosaic—natural juice contrasted with celite-treated, both filtered through a Mandler filter candle at 1/10, then diluted to 1/100 and 1/1000 for inoculation.

Character of I.F.	Dilution for inoc'n	Plants inoc'l'd	Plants dis'd	% dis'd
Natural, filtered	1/100	10	5	50
" "	1/1000	10	0	0
Celite-treated, filtered	1/100	10	9	90
" " "	1/1000	10	7	70
Control, C. T., not filtered	1/100	10	10	100

TABLE II.

Effect of time on infectivity of natural and Celite-treated infectious juice. Samples held at 1/10 during the various intervals at 6-8°C., 10 plants inoculated at each dilution, after each time-period, with each sample.

Natural I.J.			Celite-treated I.J.		
Dilution for inoc'l'n	hrs.	Diseased %	Dilution for inoc'l'n	hrs.	Diseased %
1/10	0	100	1/10	0	100
1/1000	0	90	1/1000	0	80
1/10	24	100	1/10	24	100
1/1000	24	100	1/1000	24	100
1/10	66	90	1/10	66	80
1/1000	66	90	1/1000	66	80
1/10	240	95	1/10	240	100
1/1000	240	85	1/1000	240	85

TABLE III.

Infectiousness of virus of yellow mosaic of tobacco. C.T. juice contrasted with natural juice. Virus treated at 1/1 and 1/10 dilution; 10 plants inoculated in each experiment.

Type of treatment	Dilution for inoculation	Virus treated full strength, 1/1		Virus treated at 1/10	
		Inoc'l'd immed.	Inoc'l'd after 48 hr.	Inoc'l'd immed.	Inoc'l'd after 48 hr.
Celite-treated juice	1/100	100	100	100	90
	1/1000	90	100	100	80
	1/10,000	20	20	30	50
	1/50,000	0	20	10	10
Natural juice	1/100	As control, untreated, stock virus at 1/10 dilution		100	100
	1/10,000			20	30

For comparison are given the results of one experiment with the Celite-treatment of yellow mosaic of tobacco (Table 3). Inoculations were made (a) immediately after treatment and again (b) after 48 hours, the treated and untreated juices being held for the interval mentioned in corked vials at laboratory temperature. The

irregularities recorded at the higher dilutions are not infrequent, especially after the virus has been standing.

For the present the proximate purification here proposed has found wide use in our work. This in no way minimizes the pressing importance of a highly purified virus product, if obtainable, with its attending biochemical possibilities; yet it is always possible that purification beyond a certain point may lead to inactivation.

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A Modification in the Thermostromuhr Method of Measuring Flow of Blood.

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The proper construction of the unit applied to the blood vessel is fundamental to the success of the thermostromuhr method of measuring flow of blood. Rein[1] developed the thermostromuhr and named the unit the diathermy thermo-element. This diathermy thermo-element is U-shaped and requires the use of collodion to hold it firmly on the blood vessel during an experiment. It is very satisfactory for short-time observations, but is less so for experiments in which the unit is to be kept on the blood vessel for several days.

Since it was obvious that a drop of collodion could not be expected to hold the unit to the blood vessel firmly enough to insure good electrical contact when the animal was allowed to run about, it was necessary to construct a unit better suited to such conditions. Consequently the unit described here was developed.

The dimensions of each particular unit depend on the size of the blood vessel to which it will be applied. Several units of varying sizes must be at one's disposal in order to permit the selection of the one which best fits the particular blood vessel. For a vessel the diameter of which is somewhat larger than 5 mm., the specifications for the transparent bakelite block are: length 19 mm., width

[1] Rein, Hermann, *Die Thermo-Stromuhr. Ztschr. f. Biol.*, 1928, **87**, 394. In: Abderhalden, Emil, *Handbuch der biologischen Arbeitsmethoden.* Berlin, Urban and Schwargenberg, 1929, **5**, **8**, 692.