of cellular and humoral defense mechanisms other than properdin has thus far revealed no differences which might explain the impaired rejection of implanted cells by the cancer patients.

The authors are indebted to Mr. R. W. Alvis and Dr. C. A. Doan and many of their associates for assistance and guidance in the homotransplant studies in normal volunteers.

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Received July 17, 1957. P.S.E.B.M., 1957, v96.

Preparation and Inactivation of Purified Poliovirus: Comparison of Vaccines Derived from Mahoney and Parker Poliovirus.* (23551)

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A great many studies related to preparation of poliomyelitis vaccine have been made, but little work has been done with poliovirus freed from the impurities (nutrient substances, cell components and salts) that accompany the virus in tissue culture fluids.

The advantages inherent in a vaccine derived from purified virus led us to undertake the purification of poliovirus and to study the properties of the purified virus as it became available. With respect to vaccine preparation, we were interested especially in the inactivation of the virus by formaldehyde and the immunogenic properties of the non-infective product.

Methods. Preparation of purified poliovirus. Our studies of the isolation of poliovirus led to a modified version of the Schwerdt and Schaffer procedure(1-3). Details of the isolation have been described(4,5). We have retained the first precipitation step at pH 4.0 to 4.5 with methanol in the presence of celite at 5°C. The filter cake is transferred to a column and eluted with 2% NaCl in 1% phosphate buffer at pH 7.0. This column elution results in a 300-fold increase in virus concentration. The butanol emulsification

* Portions of this paper were presented before 41st annual meeting of Am. Assn. Immunol., Apr. 17, 1957, Chicago, Ill.

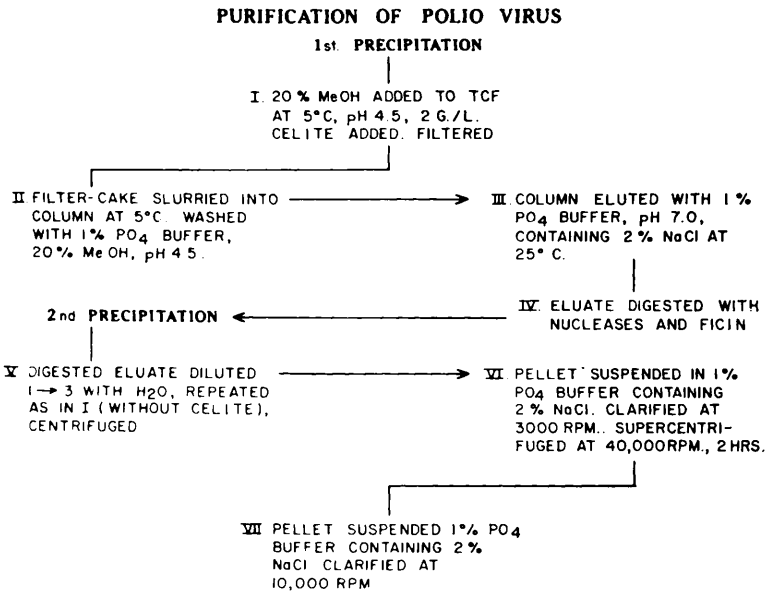

FIG. 1. Detailed flow sheet for the process used to purify large volumes of tissue culture filtrates.

step in the original procedure was abandoned in favor of digestion of non-viral protein with purified soluble ficin, a procedure which in our hands does not result in loss of virus. The digested eluate is reprecipitated and the product ultracentrifuged; pH 7.0 buffer used throughout.

Results. Yields of infectivity and complement fixing material have averaged about 30%. The method is summarized in Fig. 1. Our yields during purification were followed by modified metabolic inhibition test (M.I.T.) (6), plaque count(7) and complement fixation (CF)(8) assays; the relative accuracy and rapidity of the complement fixation method considerably expedited our studies. A comparison of the results of the 3 methods is shown in Table I.

With increasing concentration of virus, measurement of ultraviolet absorption becomes possible, and as purification proceeds the products approach the spectrum of the purified virus, as described by Schwerdt and Schaffer. The final product has a spectrum identical with that published by them(1) (Fig. 2). All 3 types of poliovirus seem to possess identical U.V. spectra.

Discussion. Some characteristics of the purified material may be mentioned. In the original tissue culture fluids (TCF) and also in the final purified products, the ratios of infective to total physical particles (specific infectivity) have ranged from 1 : 2,000 to 1 : 100,000 depending upon the history of the preparations prior to processing. The infective particles thus represent a trace within the total antigenic mass. The weight : infectivity ratios of different preparations vary widely, while the

TABLE I. Comparison of Results Obtained by 3 Assay Methods on Fractions Isolated in the Purification Process.

	M.I.T. titer ($\times 10^6$)	Plaques/ml ($\times 10^6$)	CF	Ratio: Plaques ($\times 10^6$) / CF	Yield (%)
Tissue culture fluid	6.3	13	4	3.3	100
Filtrate	1.3	2.6			
Eluate	710	960	180	5.3	53
U.C.* supernatant	870	1,560	320	4.9	
U.C.* pellet	13,000	17,000	4,800	3.5	35

* U.C. = Ultra-centrifugal.

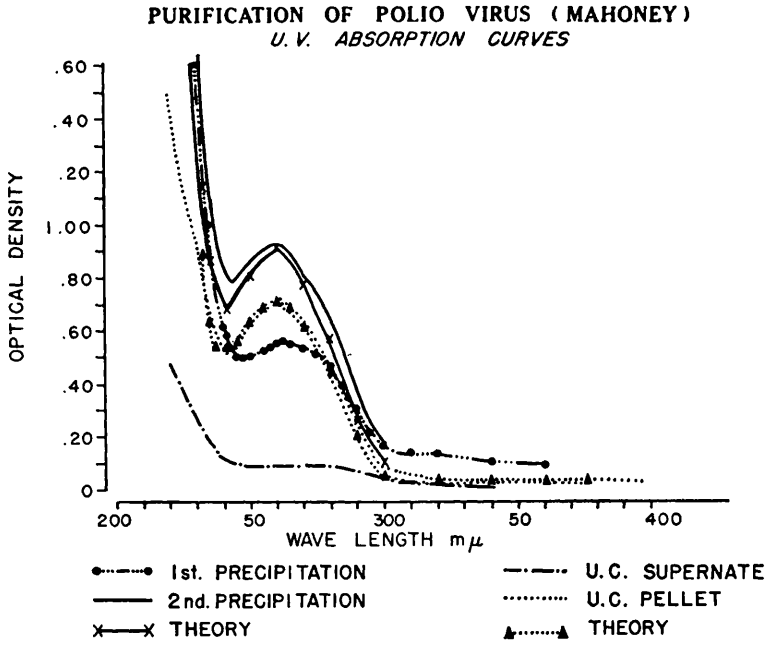

FIG. 2. Ultra-violet absorption spectra obtained on several fractions isolated in the purification process. The theoretical curves were calculated from that published by Schwerdt and Schaffer (1) for concentrations of poliovirus comparable to those prevailing in the isolated fractions.

complement-fixing ability per microgram of purified virus is approximately constant. Inactivation studies therefore reflect the behavior of that small fraction of particles which is infectious, while immunological studies relate to the behavior of the entire mass of particles, infective and non-infective.

Methods. Inactivation of purified poliovirus. The availability of purified virus has enabled us to study the effect of formaldehyde in the extinction of infectivity in such preparations. The conditions chosen were as follows: purified poliovirus was incubated at 37°C in 1% phosphate buffer, pH 7.0, in the presence of 92.5 μg/ml of formaldehyde (1 : 4,000 formalin).

Results. An experimental curve for inactivation of 20 μg/ml of purified Mahoney virus is given in Fig. 3. Examination of data obtained with Type I (Mahoney and Parker strains), Type II (MEF I strain) and Type III (Saukett strain) viruses revealed that the inactivation curves were not only similar but that, except for the difference in intercept depending upon original infectivity, they were identical. Depending upon the quantity and specific infectivity of the virus, curves of parallel slope were observed, indicating, within the precision of the assay used (metabolic inhibition test) (M.I.T.) first order kinetics. This result is to be expected, since formaldehyde is vastly in excess in the inactivation reaction, even when the purified virus is present in a concentration 300 times as great as that found in tissue culture fluids; an excess of one of 2 reactants is the classical condition for the exhibition of pseudo-first order kinetics.

Given these observations, it is meaningful to speak of the half-life of the virus. Under the conditions specified, this half-life is 1.75 hours. A correction may be applied for the inactivation of the virus at 37°C in the absence of formaldehyde, which is a slow process with a half-life of 40 hours. It must be emphasized, however, that the same kinetics cannot be expected to obtain at different pH values or in the presence of formaldehyde-binding impurities such as exist in infective tissue culture filtrates.

The first order inactivation kinetics which we have uniformly obtained are in agreement with the findings of Salk(9) but are not in accord with the recent observations of Timm *et al.*(10), Wesslen *et al.*(11) and Lycke *et*

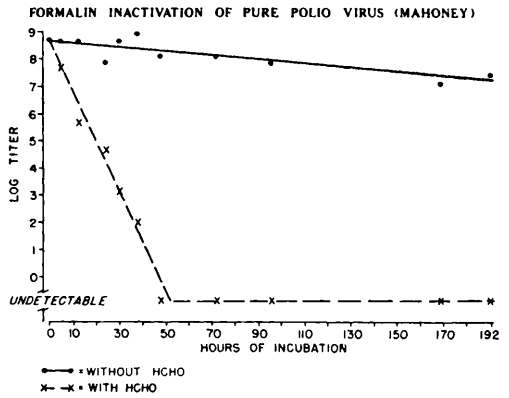

FIG. 3. Graph showing infectivity titers (M.I.T.) obtained during course of formalin inactivation of pure Mahoney virus (20 μg/ml). The inactivation was conducted at 37°C in 1% phosphate buffer (pH 7.0) with 92.5 μg/ml formaldehyde.

al.(12). The conditions used by Wesslen *et al.* and Lycke *et al.* (formaldehyde inactivation at 25°C in the presence of added glycine) are perhaps too different from ours to be usefully compared. The difference between our results and those of Timm *et al.* who observed a disproportionately large fall in infectious titer during the first few hours of inactivation in the case of partially purified virus as well as tissue culture filtrates may, however, merit comment. Apart from the possibility of the effects of differences in technic of sampling and measurement, the nature of the fluids may be decisive. Timm *et al.* note that the degree of deviation from first order kinetics varies from one virus lot to another. Bearing in mind the fact that the infective virus present in ordinary fluids represents a small surviving fraction of a much larger (and perhaps less stable) original population, it is possible that departure from first order kinetics may depend upon a high specific infectivity of the virus preparation. In support of this possibility, examination of a large number of production protocols indicates that inactivation of relatively fresh fluids frequently results in the type of deviation described by Timm *et al.* This effect is infrequent with stored fluids which, in general, show some loss of infectious titer and in which the specific infectivity has thus been lowered. In our purified virus preparations the specific infectivity has, in general, been low.

Methods. Comparison of purified Mahoney and Parker poliovirus. The immunogenic potency of some of our preparations has been determined. Our interest centered initially on the Type I component. This component is thought to be a poor antigen; its use therefore should rigorously test the capacity of a purified vaccine to immunize. The availability of purified vaccine should also permit definitive studies of the inherent antigenicity of different strains; a comparison of the Parker and Mahoney strains was made as described below.

A single lot of monkey kidney monolayer cultures was prepared. Half the flasks were seeded with Mahoney and half with Parker virus.† From the infected fluids, purified virus of each type was isolated. The titer of the Mahoney fluid was $10^{6.5}$ $TCID_{50}$/ml; that of the Parker fluid was $10^{6.3}$ $TCID_{50}$/ml. The complement fixing titer of the Parker fluid was twice that of Mahoney, and after purification, twice the quantity of purified Parker virus was isolated in comparison with Mahoney.

The purified viruses, at a concentration of 20 μg/ml, were inactivated with formaldehyde under the conditions described above. Suitable dilutions of the vaccines and the viruses from which each was derived were tested for potency in chicks.‡ Two doses were given, the first at 7 days of age and the second at 21 days of age. At 28 days, blood was obtained by cardiac puncture and antibody titers were determined by the metabolic inhibition test. Approximately 15 chicks received each dilution of vaccine and the geometric mean titers of each group of chicks were determined. Statistical analysis indicated that the log-dose response curves were linear and parallel within the limits of experimental error. The best fitting straight lines

† The authors are indebted to Mr. Robert Rotundo and associates in the Biological Production Laboratories, Merck Sharp & Dohme Division for supplying these materials.

‡ The authors gratefully acknowledge the cooperation of Dr. J. E. Prier and his co-workers of the Biological Development Laboratories, Merck Sharp & Dohme Division, in conducting the chick test.

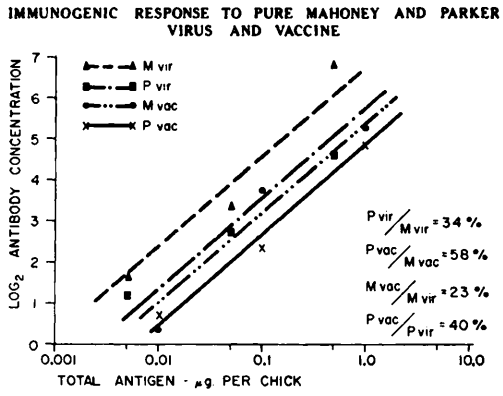

FIG. 4. Dose response curves based on data obtained in chick potency test.

were calculated by the method of least mean squares assuming a common slope.§ The log-dose response curves are shown in Fig. 4 where the logarithm (base 2) of the antibody titer is plotted against the logarithm (base 10) of the dose.

Results. The vaccine derived from Parker virus was 58% as potent as that derived from Mahoney; this difference was found not to be significant. The parent infective viruses differed significantly; Parker virus was 34% as potent as Mahoney. In this connection the 2-fold yield of Parker with respect to Mahoney should be considered; if the potency were to be calculated taking into account the original concentrations of the 2 viruses in the tissue culture fluids, or the yields of purified virus isolated, these differences would disappear. The vaccines were less potent than the viruses from which each was derived. Parker vaccine was 40% and Mahoney vaccine 23% as potent as the parent viruses from which they were prepared. These differences, although small in relation to the precision of the chick potency assay, were found to be significant.

Summary. 1) Purified virus was prepared from tissue culture fluids containing Type I (Mahoney and Parker strains), Type II (MEF I strain) and Type III (Saukett strain) poliovirus. 2) The purified viruses were successfully inactivated with formaldehyde and the inactivation kinetics studied. 3) Purified Type I (Mahoney and Parker strains) viruses and the vaccines derived from them were shown to be immunogenically potent in chicks. 4) No statistical difference was found in the immunogenic potency of purified Parker and Mahoney vaccines in the chick. 5) Purified infective Mahoney virus was found to be significantly more potent than Parker virus. 6) Vaccines derived from purified Mahoney and Parker viruses were significantly less potent than the parent viruses.

§ We wish to express our thanks to Mr. Joseph L. Ciminera, Biometrician, for evaluating the results of the chick test.

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