the data of Ragan & Bordley [q] and proposed that from 5 to 25 mm Hg, depending on arm thickness, should be subtracted from both systolic and diastolic indirect pressure readings on stout arms in order to exclude the error.

The error introduced by using too narrow a bag is more marked the thicker the arm.[c, l] In some of the above studies cuffs too short to encircle the arm have been used; in some reports the cuff length has not been mentioned. The use of cuffs of varying length may, at least partly, explain the controversial observations about the effect of arm thickness on the error of the indirect method.

A correlation between arm circumference and indirect blood pressure readings may depend on two factors: (*a*) the frequent real occurrence of elevated intra-arterial pressures in the obese, and (*b*) a technical error introduced by the cuff. Dr W. Holland [z] has given the preliminary information that in a study made in collaboration with Dr Humerfelt, the difference in systolic pressure, as measured with the direct and indirect methods respectively, appears to increase by 6 mm for every inch of arm circumference. However, in our studies with the large cuff, there was no correlation between the blood pressures and the arm circumference.[m, n] Similarly, there was no tendency to increasing error with thicker arms.[o] However, obesity was poorly represented in both series. Further comparisons of intra-arterial and indirect measurements on obese subjects will be needed in order to demonstrate whether the use of a cuff of the recommended size will entirely exclude systematically elevated indirect blood pressure readings in obese subjects.

Conclusion

The tentative conclusion is that a cuff containing a rubber bag 14 cm wide and long enough to encircle the arm—as recommended in the report of the WHO Expert Committee on Cardiovascular Diseases and Hypertension [k]—gives a close approximation to the intra-arterially recorded systolic and diastolic pressures, without any correction for arm circumference, at least within the range of arm sizes generally met. Such a cuff has proved to be better than commercially available cuffs with smaller bags.

[z] Personal communication, 1961.

Cultivation of Pure Influenza Virus in Chorio-allantoic Membrane Cultures *in Vitro*

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Employing the glass wool technique and the medium V developed by the author, Veeraraghavan et al. [a] described a method for the cultivation of influenza virus in chorio-allantoic membrane (CAM) tissue cultures *in vitro*. The method is found equally satisfactory for cultures in volumes ranging from 1 ml to 350 ml and is as sensitive as the egg for the titration of different strains of influenza virus and their neutralizing antibodies. Relatively pure virus for vaccine production and complement-fixing antigen for diagnostic purposes can be produced in large volumes with ease and economy.

Studies on the purity of the culture virus showed that its nitrogen content varied from 0.1832 to 0.2832 mg per ml while that of infected allantoic fluid ranged between 0.6561 and 0.7131 mg per ml. The nitrogen content of the virus purified by ultracentrifugation and made up to the original volume of the allantoic fluid with medium V varied from 0.1196 to 0.1416 mg per ml, the nitrogen content of the medium being 0.04 mg per ml. Since the nitro-

[a] Veeraraghavan, N., Kirtikar, M. W. & Sreevalsan, J. (1961) *Bull. Wld Hlth Org.*, **24**, 711.

gen content of the culture virus was higher than that of the ultracentrifuged material experiments were undertaken to enhance its purity.

Methods

Elimination of tissue nitrogen. Preliminary investigations showed that the higher nitrogen content of the culture virus was due partly to the inadequacy of the method used for washing the CAM [a] and partly to the tissue nitrogen from the CAM liberated into the medium during rolling. It was found possible to get rid of a part of the tissue nitrogen by the following method.

The culture containing CAM on glass wool and the medium V in the proportion mentioned was put up as described [a] and rolled for three hours at 37°C. The medium was removed and the CAM washed with two changes of Hanks' balanced salt solution without glucose (BSS) adjusted to pH 7.1 and pre-warmed to 37°C. The requisite volume of fresh, pre-warmed medium V, adjusted to pH 7.1, was then added, followed by the virus. The bottle was then rolled by hand for five minutes to mix the contents and then in a roller drum revolving at nine revolutions per hour at 37°C for 60-72 hours, when the highest titres of the virus were obtained. By this procedure it was possible to obtain water-clear cultures of influenza virus, whose purity as judged by tissue culture infective doses ($TCID_{50}$) per mg of nitrogen was as good as the material purified by ultracentrifugation.

This procedure, however, was considered rather uneconomical in view of the large quantities of medium V that had to be used for the preliminary rolling of CAM on glass wool. Experiments undertaken to determine the constituent of the medium V responsible for the elimination of nitrogen indicated that it was glycerol. Controlled investigations showed that preliminary rolling of CAM with BSS containing 0.4% glycerol (Analar) for three hours at 37°C gave consistently good results, although the final virus yields tended to be slightly lower. It was found that washing the CAM with two changes of BSS could be dispensed with provided the pH of the medium V added was adjusted to 7.3 instead of 7.1. Another improvement found beneficial was to roll the glass wool in the culture bottles with sterile, double-distilled water containing penicillin and streptomycin for one hour at 37°C prior to the addition of glycerinated BSS. This removed the traces of alkalinity which tended to persist in the glass wool in spite of careful acid treatment and washing.

Improvement of the medium. It is obvious that the above method, which is more economical, should prove very useful if the virus titres of the cultures could be stepped up. Attempts were therefore made to improve the medium.

Of the various compounds tried it was found that the addition of L-glutamic acid and L-aspartic acid in a concentration of 10 mg and 5 mg respectively per 100 ml of the medium V gave consistently good results. The improved medium was designated as medium V1. The stock medium V1 was prepared as follows.

Sodium pyruvate, 2 g; L-aspartic acid, 50 mg; L-glutamic acid, 100 mg; glycine, 50 mg; sodium molybdate, 50 mg; folic acid dissolved in minimum quantity of 1% sodium bicarbonate, 4 mg; glycerol (Analar), 4 ml; and double-distilled water to 100 ml. The medium was sterilized by filtration through a Ford Sterimat GS pad. As the pads were generally alkaline they were washed with double-distilled water before sterilization. The medium was stored at −20°C. When required, concentrated medium was diluted tenfold with BSS, adjusted to pH 7.3 with sterile 5% sodium bicarbonate and used.

Improved method of putting up cultures. The sterile bottle containing glass wool was rolled for one hour at 37°C with sterile, double-distilled water containing penicillin and streptomycin. The volume of distilled water used was the same as that of the culture. The water was removed and replaced with an equal volume of BSS containing 0.4% glycerol adjusted to pH 7.3. The minced CAM from 13-day-old chick embryos, washed three times with BSS,[a] was resuspended in an equal volume of medium V1. This suspension was added to the glycerinated BSS in the proportion of 0.07 ml per ml of the final volume of the culture. The bottle was rolled on its side slowly to make the CAM adhere to the glass wool and form a sheet and then transferred to the roller drum at 37°C for three hours. The glycerinated BSS was then removed and replaced with an equal volume of freshly prepared, pre-warmed medium V1, adjusted to pH 7.3. After addition of the virus, the culture was rolled for 60-72 hours at 37°C. When tube cultures were used for titration, the preliminary rolling was not done. The method was the same as that described previously [a] except that medium V1 was used instead of medium V.

COMPARATIVE VALUES OF DIFFERENT STRAINS OF INFLUENZA VIRUS GROWN IN MEDIUM V AND VI

Strain	Medium V					Medium V1				
	HA/ml	Log_{10} $TCID_{50}$/ml	Log_{10} $TCID_{50}$/HA	Log_{10} $TCID_{50}$/ mg N	CF	HA/ml	Log_{10} $TCID_{50}$/ml	Log_{10} $TCID_{50}$/HA	Log_{10} $TCID_{50}$/ mg N	CF
PR8	512	8.30	5.59	9.19	32	2 048	9.50	6.19	10.37	32
FM1	256	7.50	5.10	8.40	32	256	9.15	6.74	10.04	32
PAR	128	7.31	5.21	8.20	32	256	9.15	6.74	10.02	32
Gilbert (LEP)	128	7.50	5.40	8.40	32	512	9.70	6.99	10.56	32
Crawley	128	7.31	5.21	8.20	8	256	9.15	6.74	10.01	16

Results

The results obtained with antigenically representative strains of influenza virus using the modified method and the medium V and V1 are given in the table. For these comparative studies, 80-ml cultures were put up in 250-ml Pyrex bottles containing glass wool, and the 72-hour cultures were titrated. The methods used for the haemagglutination (HA), complement-fixation (CF) and $TCID_{50}$ titrations were the same as those described previously.[a] Five tubes per dilution were used for determining the $TCID_{50}$ of the cultures. The viruses studied were (*a*) PR8, (*b*) FM1 (type A1), (*c*) PAR (non-avid A2), (*d*) Gilbert (avid A2) at low (3rd) egg passage (LEP), and (*e*) Crawley (type B).

Comments

The HA and $TCID_{50}$ per ml titres obtained with medium V1 are generally greater than those obtained with medium V. The high $TCID_{50}$/HA values indicate that in each case complete virus is being produced. The results are similar when the strains are grown in 350-ml volumes.

The $TCID_{50}$ per mg of nitrogen values show that the culture viruses obtained with medium V1 are purer than those obtained with medium V and as good as those purified by ultracentrifugation.

The culture using medium V1 is also found to be more sensitive than that employing medium V for purposes of titration of different strains of virus and their antibodies.

The pure virus obtained by this method should prove useful for purposes of live virus immunization against influenza.

* * *

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Intradermal and Fluorescent Antibody Tests on Humans Exposed to *Schistosoma bovis* Cercariae from Sardinia

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Simple, economical and reliable tests are required in order to establish the prevalence of schistosomiasis in large population groups. Intradermal tests are available and have been used with varying degrees of success in many of the world's endemic areas (Kagan, 1958; Pellegrino, 1958; Sadun et al., 1958; Hunter et al., 1958; el Azzawi & Klimt, 1958; Manson-Bahr, 1958; Hsu, 1959; Sadun et al., 1959; Pellegrino,