Cardioactive and vasoactive drugs as aids to diagnosis (*)

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The possibility of surgical correction of congenital abnormalities and acquired valvular lesions has increased the interest of auscultation as one of the most important diagnostic methods. Even though cardiac catheterization and angiocardiography are often used in the final evaluation of certain cases, not all patients are submitted to these procedures. Therefore, auscultation and its technical counterpart (phonocardiography) have basic importance among the physical and technical diagnostic methods.

Auscultation supplies information concerning loudness of heart sounds, splitting of heart sounds, presence of additional systolic or diastolic sounds, and the characteristics of murmurs. All these data are usually collected while the patient is at rest and cardiac output is at a relatively low level. It is obvious that the heart sounds and the murmurs are modified by changes in heart rate, blood pressure and blood flow. This is why several drugs have been employed in the study of murmurs in order to improve the accuracy of diagnosis and for their differential diagnosis.

Effect of drugs on hemodynamics and changes of murmurs resulting from their action (Tables 1, 2, 3)

Digitalis.

Digitalis increases the contractility of the heart and this is evidenced by an increase of rapidity and magnitude of left ventricular contraction. It decreases the automaticity of the heart and stimulates the vagus, thus prolonging ventricular diastoles. It increases cardiac output and decreases venous pressure in patients with congestive failure.

The changes of cardiac dynamics caused by digitalis should result in an increase of all systolic murmurs, both in congenital and in acquired valvular

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TABLE I.

Conditions causing murmurs in the left heart

Condition	Type of murmur	Digitalis	Amyl nitrite (late)	Methoxamine	Isoproterenol
Mitral insufficiency (organic)	Pansystolic	+	—	+	
Mitral stenosis (organic) .	Diastolic-presystolic	—	+	—	
Mitral stenosis (relative) .	Diastolic-presystolic	—	—	+	
Aortic insufficiency . . .	Early-diastolic	+	—	+	
Aortic stenosis (valvular) .	Systolic, ejection type	+	+—	—	+
Aortic stenosis (muscular)	Systolic, ejection type	++	+++	— —	+++
Aortic stenosis (relative) .	Systolic, ejection type	+	+	—	+

diseases while diastolic murmurs are variously affected. The full effect of digitalis on murmurs is usually obtained after a few days of treatment unless large doses are administered intravenously. This drug is not currently used for functional testing.

In detail, the pansystolic murmur of organic mitral insufficiency should be increased by digitalis. On the other hand, that of « relative » mitral insufficiency might have a variable behavior. The pansystolic murmur of organic tricuspid insufficiency should be increased by digitalis but that of relative tricuspid insufficiency should be decreased, due to better left ventricular function which is followed by decreased pulmonary and right ventricular pressures.

The murmur of mitral stenosis (organic or relative) is decreased by digitalis on account of longer diastoles that cause a less tumultuous flow through the mitral valve and lower left atrial pressure. The same is true for tricuspid stenosis. The terms « relative » mitral or tricuspid stenosis applies to those « functional » diastolic or presystolic murmurs which occur during overload of one ventricle. Thus « relative » mitral stenosis is encountered in left ventricular failure, myocarditis, aortic insufficiency (A. Flint murmur), or mitral insufficiency. « Relative » tricuspid stenosis is observed in chronic or acute pulmonary heart disease, mitral stenosis, tricuspid insufficiency, or atrial septal defect.

In regard to basal murmurs, the murmur of aortic insufficiency should be increased by digitalis because the stronger contraction elevates aortic systolic pressure. The longer duration of diastole should make the murmur more

discernable. The murmur of aortic stenosis should also be increased because of stronger left ventricular contractions (*). The same applies to pulmonary insufficiency and stenosis, with the exception of the diastolic murmur of « relative » pulmonary insufficiency (G. Steell murmur), which should be decreased by digitalis due to lowering of pulmonary arterial pressures.

TABLE 2.

Conditions causing murmurs in the right heart

Condition	Type of murmur	Digitalis	Amyl nitrite (early)	Isoproterenol
Pulmonary insufficiency . (organic)	Early-diastolic	+	+	
Pulmonary insufficiency . (relative)	Early-diastolic	—	+	
Pulmonary stenosis . . . (valvular)	Systolic, ejection type	+	+	+
Pulmonary stenosis . . . (muscular)	Systolic, ejection type	+ +	+	+ + +
Pulmonary stenosis . . . (relative)	Systolic, ejection type	+	+	+
Tricuspid insufficiency . (organic)	Pansystolic	+	+	+
Tricuspid insufficiency . (relative)	Pansystolic	—	+ —	+
Tricuspid stenosis . . . (organic)	Diastolic-presystolic	—	+	
Tricuspid stenosis . . . (relative)	Diastolic-presystolic	—	+	

In congenital shunts, digitalis should increase the murmur of ventricular septal defect and that of patent ductus. However, if there is pulmonary hypertension, these murmurs might decrease. Digitalis should not affect the murmur of atrial septal defect.

The above considerations on the probable effect of digitalis glycosides were based on the physiologic effects of these substances. We have recently initiated a program of study on the behavior of murmurs during and after

(*) The systolic murmur of subaortic muscular stenosis is increased more than that of valvular aortic stenosis.

an intravenous injection of ouabaine (0.25 mg). With surprise we have noted that all murmurs originating in the left heart decrease after ouabaine, with a peak of action at 20-30 min from the injection. This is true, not only for

TABLE 3.

Congenital shunts causing murmurs

Condition	Type of murmur	Digitalis	Amyl nitrite (early)	Methoxamine	Isoproterenol
Atrial septal defect	Systolic, ejection type				
Ventricular septal defect	Pansystolic				
Ventricular septal defect with pulmonary hypertension	Pansystolic or ejection type				
Tetralogy of Fallot	Systolic				
Patent ductus arteriosus	Continuous				
Patent ductus with pulmonary hypertension	Systolic				

the murmur of mitral stenosis, but also for that of mitral insufficiency, that of aortic insufficiency and that of aortic stenosis.

The study is continuing and should be concluded within one year.

Amyl nitrite.

Amyl nitrite causes both systemic and pulmonary vasodilatation. It decreases arterial systemic pressure; it increases velocity of aortic flow and rapidity of left ventricular ejection; it increases the heart rate through a carotid sinus reflex. As a result, venous return to the right heart is increased within the first 30 to 60 sec while that to the left heart is increased after about 75 to 90 sec.

The pansystolic murmur of organic mitral insufficiency is decreased in the early phase by amyl nitrite. That of «relative» mitral insufficiency may either decrease or increase. The diastolic murmur of organic mitral stenosis is increased by amyl nitrite, largely on account of shorter diastoles causing higher left atrial pressure and more tumultuous flow through the mitral valve. In contrast, the murmurs caused by «relative» mitral stenosis (murmurs of C. Coombs, of A. Flint, of myocarditis, of left ventricular failure) may decrease, due to decrease of left ventricular pressures. However, this change is not always found, especially if there is left ventricular failure.

Even the lack of increase of the murmur during the phase of tachycardia caused by amyl nitrite may be in favor of a functional diastolic rumble. The systolic murmur of valvular aortic stenosis may either increase or decrease while that of muscular subaortic stenosis generally increases.

The action of this drug on murmurs of the right heart is of particular interest.

The blowing diastolic murmur of pulmonary insufficiency, whether organic or relative (G. Steell), is usually increased by amyl nitrite within the first 15 to 30 sec due to greater venous return and greater pulmonary flow. The harsh, ejection-type, systolic murmur of pulmonary stenosis, whether organic or relative (pulmonary flow murmur including that of some innocent murmurs), is also increased by amyl nitrite through the same mechanism. The systolic murmur of tricuspid insufficiency (organic or relative) and the diastolic murmur of tricuspid stenosis (organic or relative) are generally increased by amyl nitrite in an early period.

In regard to congenital shunts, here again amyl nitrite has a significant diagnostic value. The systolic, ejection-type, pulmonary flow murmur of atrial septal defect is increased while the pansystolic murmur of ventricular septal defect is decreased. In ventricular septal defect with pulmonary hypertension, the murmur behaves like in any pulmonary flow murmur and therefore increases. In tetralogy of Fallot, the murmur bahaves as in ventricular septal defect and therefore decreases. In regard to patent ductus, the uncomplicated form shows a decrease of the murmur while that associated with pulmonary hypertension may either decrease or increase.

Methoxamine (*Vasoxyl*).

Methoxamine causes a constriction of the systemic resistance vessels and an increase of left ventricular systolic pressure. Left ventricular diastolic pressure is often also increased by large doses of this drug. The rapidity of left ventricular contraction is decreased.

The systolic murmur of mitral insufficiency (organic or relative) is increased by the rise of left ventricular systolic pressure. The diastolic-presystolic murmur of organic mitral stenosis is decreased by the bradycardia that causes a lowering of left atrial pressures. On the contrary, that of « relative » mitral stenosis is increased by the left ventricular overload caused by methoxamine. In cases with atypical systolic murmurs, the murmur usually becomes more typical and easier to diagnose. The murmur of valvular aortic stenosis is decreased by methoxamine, and the same is true in « relative » aortic stenosis (aortic flow murmur). The murmur of muscular subaortic stenosis is also decreased by methoxamine but to a greater extent because the increased resistance tends to decrease the gradient between inflow and outflow tracts of the left ventricle.

The pulmonic systolic flow murmur of atrial septal defect increases in some cases. The pansystolic murmur of ventricular septal defect is constantly increased (increase of left ventricular pressures). An exception seems to occur when the ventricular septal defect is associated with pulmonary hypertension. In such cases, the murmur has been found to decrease by some authors.

The systolic murmur of tetralogy of Fallot is generally increased due to bilateral ventricular hypertension that increases the flow through the stenotic pulmonary valve.

The murmur of patent ductus is increased by methoxamine, except in cases with pulmonary hypertension where either increase or decrease may be observed.

It is interesting to mention that, while in most cases of pulmonary insufficiency (whether organic or relative) the blowing diastolic murmur is not affected by methoxamine, a marked increase of the murmur is noted in cases of Eisenmenger's syndrome. In the latter, the increase of aortic pressure causes an increase of pressure in both ventricles, an increased flow through the pulmonary valve, and an increased regurgitation.

Epinephrine (*Adrenaline*).

Epinephrine causes tachycardia, an increased rapidity of left ventricular contraction, and an increase of cardiac output. Even though it causes peripheral vasodilatation, cardiac output is so augmented that left ventricular and aortic systolic pressures are increased.

The changes caused by epinephrine are less typical than those caused by other drugs due to increase of both left ventricular energetics (similar to that caused by isoproterenol) and aortic pressure (similar to that caused by methoxamine). For this reason, epinephrine is not currently employed in our laboratory as a diagnostic test.

Norepinephrine (*Nor-adrenaline*).

Norepinephrine has chiefly a peripheral effect causing constriction of both the resistance and the capacitance vessels. It is not usually employed as a diagnostic test but the changes caused by this drug would be similar to those caused by methoxamine.

Isoproterenol (*Isuprel*).

Isoproterenol increases the rapidity and power of contraction of the myocardium. It causes tachycardia, shorter systoles, and shorter diastoles. It causes dilatation of the systemic resistance vessels and also of the pulmonary vessels. Thus it causes greater flow, higher systolic pressure, and lower diastolic pressure in both the pulmonary artery and the aorta.

While isoproterenol increases the systolic murmur of valvular aortic stenosis, of muscular aortic stenosis and of « relative » aortic stenosis (aortic flow murmur), the increase is greatest in muscular stenosis. For this reason, the use of this drug has diagnostic value for the clinical recognition of this form of obstruction. A similar behavior is encountered on the right side because the systolic murmur of muscular subpulmonic stenosis is enhanced much more than that of valvular or « relative » stenosis.

Differential diagnosis

Certain diagnostic possibilities are not aided by the use of drugs. Thus, the pansystolic murmur of ventricular septal defect is affected in the same way as that of mitral insufficiency and that of the tetralogy of Fallot.

TABLE 4.

Differential diagnosis

	Mitral stenosis (organic)	Mitral stenosis (relative)	Mitral stenosis (organic)	Aortic insufficiency	Aortic insufficiency	Pulmonary insufficiency	Pulmonary stenosis	Ventricular septal defect	Tetralogy of Fallot	Muscular pulmonary or aortic stenosis	Valvular pulmonary or aortic stenosis
Amyl nitrite	+	—	+	—	—	+	+	—	—		
Methoxamine	—	+	—	+	+			+	+		
Isoproterenol										+—	+++

The following differential diagnoses, on the other hand, are currently aided by drug tests (Table 4).

1) *Organic mitral stenosis versus relative mitral stenosis.* — The diastolic rumble is increased by amyl nitrite in the organic stenosis ; it may decrease (or at least fail to increase) in relative stenosis (Austin Flint and other functional diastolic murmurs). Methoxamine decreases the murmur of organic stenosis, increases that of relative stenosis.

2) *Mitral stenosis versus aortic insufficiency.* — The diastolic murmur is increased by amyl nitrite in mitral stenosis, decreased in aortic insufficiency. On the contrary, methoxamine decreases the murmur of mitral stenosis while it increases that of aortic insufficiency.

3) *Aortic insufficiency versus pulmonary insufficiency.* — The diastolic murmur is decreased by amyl nitrite in aortic insufficiency, increased in

pulmonary insufficiency. Methoxamine increase the murmur of aortic insufficiency, does not change that of pulmonary insufficiency.

4) *Pulmonary stenosis versus ventricular septal defect or tetralogy of Fallot.* — Amyl nitrite increases the murmur of pulmonary stenosis, decreases that of the other two conditions. Methoxamine increases the murmur of ventricular septal defect as well as that of the tetralogy.

5) *Aortic or pulmonary muscular stenosis versus aortic or pulmonary valvular stenosis.* — The murmur is increased by isoproterenol in all these forms of stenosis ; however, the increase is much greater in the muscular than in the valvular form. A similar result is obtained with digitalis.

6) *Organic versus relative pulmonary insufficiency.* — In organic pulmonary insufficiency, digitalis causes an increase of the murmur by augmenting the power of right ventricular contractions. On the contrary, in « relative » pulmonary insufficiency, digitalis often decreases the murmur. This is particularly true of the relative pulmonary insufficiency caused by left heart obstruction (mitral stenosis) or left heart failure through decrease of left atrial and pulmonary pressures. It may not occur if the insufficiency occurs in pulmonary heart disease where the pulmonary pressure is less affected. Methoxamine increases the murmur of pulmonary insufficiency only in Eisenmenger's syndrome while it does not change that of other forms, whether caused by organic or relative insufficiency.

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The need for a standardised cell substrate for virus vaccine production (*)

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During the last 15 years there have been such enormous advances made in virology, largely due to the development of cell culture techniques, that it is pertinent for us to take stock of the significant milestones and enquire into the future.

Fifty years ago the only three virus vaccines used were prepared in or on living animals. Smallpox virus was grown on the skin of animals and the vaccine made from the lymph scraped from the infected tissues. Rabies virus was grown in the brains of susceptible animals and the vaccine consisted of the virus killed by phenol in the presence of the brain tissue. Yellow fever virus was grown in fertile eggs from hens and the vaccine made from the infected embryos. No tests were done on the animals or eggs used in the production of these vaccines and indeed little was known about the viruses infecting or inherently endemic in the birds and animals. Although some small modifications have been made in production methods, there has been little change in these vaccines to the present day.

When a vaccine against poliomyelitis was urgently required there was no known easily available susceptible laboratory animal and the production of this vaccine had to await further technical developments. There were two major advances made at about the same time. Enders, Robbins and Weller placed the growth of cell cultures *in vitro* on a firm footing and showed that poliomyelitis virus could be grown in the tissue on a commercial scale. This was indeed a significant development but it would not have been of such universal application if penicillin, discovered by Fleming, Chain and Florey, had not so successfully suppressed the bacterial contaminants that had bedevilled tissue culture for so many years. The tissue chosen for poliomye-

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litis vaccine production was grown from monkey kidneys and the eradication of the disease in many countries is now history. The developments in virology, however, as a result of the opening of new frontiers in tissue cultures have not only shown the presence of hitherto undetected viruses, but they have also shown the effects of both virus/cell and virus/virus interaction. The present findings demand the use of a virus free substrate so that there shall be no possibility of interaction with the vaccine virus. It is also essential to avoid the possibility of an endemic infectious agent in the vaccine escaping detection by our safety tests.

The modern trend has been to use cell cultures prepared from tissue of clean animals or birds. Thus, measles virus is grown on chick cell fibroblasts derived from the fertile eggs of chickens known to be free from and continuously monitored for the presence of fowl leucosis viruses. Measles vaccine is also prepared on kidney tissue obtained from dogs bred under clean conditions. Furthermore, rubella vaccine is currently being prepared in cell cultures from dog kidneys, rabbit kidneys and duck embryos. Although such steps to obtain a cleaner substrate must be encouraged, they cannot be regarded as the ultimate answer. One strain of rubella virus is grown in human embryo fibroblast cells propagated in series, which is a much cleaner tissue than any primary tissue.

When we look at the enormous developments that have taken place in virus vaccine production it is surprising that so little attention has been given to the standardisation of the cell substrate. There is a wealth of information on the growth requirements of different cell cultures and much has been done to obtain reproducibility between batches of calf serum. The virus used for vaccine production is selected with the utmost caution requiring carefully controlled clinical trials to ensure that it is both safe and immunogenic. Indeed the virus seed pool used as the starting point for the production of each batch of vaccine is thoroughly checked to ensure its freedom from extraneous agents and untoward characteristics. Finally, each batch of vaccine undergoes the most stringent tests to ensure its safety. It seems logical to suggest that just as much care and attention should be given to the selection, testing and standardisation of the cell substrate used for the propagation of the virus to be incorporated in the vaccine.

What are the alternatives to primary monkey kidney tissue ? Several have been suggested : Earle's mouse L cells, continuous monkey kidney cells and even chick embryo fibroblast cell lines. In making a choice several conditions must be satisfied.

1. The cell must remain normal throughout life.
2. It should be embryonic tissue since these are the least likely to be inherently contaminated.

3. It should be tissue from a non-differentiating organ.
4. It must be a tissue undergoing a sufficient number of cell doublings to provide an economic source of tissue to the manufacturer.
5. It should be a tissue susceptible to most human viruses.

Such a cell line was started by Professor L. Hayflick and Dr. P. Moorhead when they established a population of human embryo lung fibroblast cells. One such cell line is known as WI-38. Many laboratories have examined this tissue and none has found a contaminating virus, whereas all have found the cells to remain normal throughout 30 to 35 cell doublings. We have learnt a great deal about the practical limits of handling such cells so that we are now in a position to propose recommendations to those wishing to establish their own cell line.

In general the criteria are concerned with obtaining uncontaminated tissue from a normal person, proving its normality throughout its finite life and showing that there are not likely to be extraneous agents or untoward reactions occurring in use. Many of the tests may seem somewhat tedious but there are no tests proposed for a new cell substrate that have not been satisfied already by the WI-38 cell line.

History and genealogy of the cell line.

It is most important to know the history of the cell line and the data required include the age and sex of the donor. Hayflick started with foetal tissue because this was most likely to be free from contaminants. The possibility of the presence of extraneous agents would be much reduced by the placental barrier and taking the foetus by caesarian section would still further prevent contamination by external sources. A further comment on the source of tissue was made by Hayflick who purposely chose non-functional cells and avoided the use of kidney because of its likelihood of being a reservoir for latent viruses. This reduced the choice to skin, muscle, lung etc. and the choice of lung in the case of WI-38 was arbitrary, except that it was an inner organ protected from contamination by the external skeleton.

The sex of the donor is merely to act as a marker in the event of cross contamination of the cell line since the sex chromosome can be detected so easily. Knowing the sex of the donor, however, is required more for having a complete record than for any scientific reason. Where applicable it is important to have a full and detailed family history of the donor, particularly with respect to congenital abnormalities or genetic defects, absence of evidence of neoplastic disease in the donor, parents and living siblings at the time the cell line is established. It is appreciated that these data

are particularly applicable for the establishment of a human cell line, but even if an animal cell line is being considered it is essential to have an accurate record of the health of the stock.

Finally, in this section it is necessary to have the full record of the cultural history of the cell line including the method used to establish the initial outgrowth of cells from the tissue pieces.

Culture media.

There are many culture media used for cell growth, some of which are more toxic than others. Parker always considered his 199 medium to be too toxic for optimal growth of cells and proposed its replacement by 1066 medium, but the virology world did not make the change. Eagle, Earle, Guy have all put forward satisfactory balanced salt solutions and when these are supplemented with amino acids, vitamins, nucleotides and serum, a complete medium is obtained. The medium used for the initial outgrowth of the cells from the tissue pieces is the best for the continued propagation of the cells. For this reason, it is most important that an accurate description of the medium composition is recorded, since a sudden change of medium during propagation may cause chromosome abnormalities. It is also important to have a large batch of fully tested serum in stock since deficiencies or toxicities of serum may also cause changes in the karyology. A serum has not been fully tested until it has been used to support the growth of the cells for at least 5 cell doublings. It is of interest to learn that a new sterile powdered Eagle's medium incorporating sterile serum and fully standardised before use will be available in the next year.

Growth characteristics.

The time taken for cell doublings as well as the number of doublings occurring in the finite life of the cell line are critical characteristics. An infection of the cells most often destroys the cells or causes them to grow more slowly. Furthermore, a transformation of the cell may occur by infection with an oncogenic virus and although this may not cause a change in cell doubling time it will certainly give the cell indefinite life. Both these parameters are good markers, therefore.

Freezing of cell seed.

The greatest advantage in the use of a cell line for virus vaccine production is the facility to freeze the cell population indefinitely in small aliquots until such time as a sample is exhaustively examined and shown the cell population to be free from extraneous agents and untoward reactions.

It is important to freeze the cells in such a manner that the highest proportion survive both the freezing cycle and the thawing process. It is necessary to protect the cell from damage during the freezing and this is done with glycerol or dimethyl sulphoxide $(CH_3)_2 \cdot SO$. There are no real advantages of one over the other, but we find that with DMSO there is a higher survival rate of cells.

The cells must be cooled for the first 20°C to 30°C at a rate of 1° per minute, after which they may be plunged into a very cold temperature. The highest temperature at which they should be stored is —70°C, but they will store for much longer periods of time in either liquid nitrogen (—190°C) or the gas phase of a liquid nitrogen container. Certainly the half life of cells is significantly decreased by every degree above —70°C until at —50°C the half life is as short as three weeks.

Just as much attention should be paid to the thawing of the cells as to the freezing. Cells frozen in ampoules in liquid nitrogen should always be handled with care and a face vizor must be used in case the ampoules explode. As soon as it is removed from the liquid nitrogen the ampoule should be immersed in warm alcohol and thawed rapidly. Alcohol is used so that the outside of the ampoule will remain sterile and contamination during the transfer of the thawed cells to a growth bottle will not occur.

Karyology.

At the onset it should be made clear that a normal karyology does not necessarily free a tissue from having any untoward properties. The importance of monitoring cells by karyological studies, however, is to show that there has been no dramatic changes during propagation. Furthermore, the sampling of cells for studies is a compromise between the ideal and the practical. These examinations are extremely time-consuming and tedious; a fully trained karyologist has to spend many hours at this routine work and much thought has been put into the cell sample so that a realistic answer of the normality of the cells will be obtained without an excessive amount of work.

A certain amount of discrepancy between laboratories has arisen as a result of using different criteria of abnormalities. The criteria of acceptability for the various analyses are shown in Table 1. The limits of acceptability have been selected as a result of many years observation and it is known that the WI-38 cells, for example, can meet these requirements. It is worth emphasising that the suggested limits of abnormality above which the cell substrate must be rejected are based on frequencies of abnormality that may occur. It is merely a very sensitive check that the cell has remained normal throughout propagation.

TABLE 1.

Criteria of acceptability based on karyological analyses.

Examination	No. of cells taken	Observations
1. Exact counts	100 metaphase cells	> 2 % 2n + 1 + 2 or + 3 etc. not acceptable
2. Analysis of karyotype	20 of the above cells in (1)	Photographic reconstruction should show cells normal
3. Chromosome breaks and gaps	100 cells in (1)	Tabulated as open break achromatic gaps, etc.; should not be more than 2 % 2.4 % to 9.4 %
4. Structural chromosomal abnormalities . .	100 cells	Unstable structural abnormalities (dicentrics, rings, exchange configuration, quadriradials, etc.) stable structural abnormalities (deletions, inversions, reciprocal translocations) These should not exceed 1 %
5. Polyploidy	300 unselected metaphase cells	Normal range about 1 % to 5 % if upper statistical limit (P = 0.025) is > 10 % reject cells

Tests for freedom from adventitious agents.

It is understandable that any new cell substrate suggested for use on a large scale must be thoroughly checked for the presence of adventitious agents. Thus any system uniquely capable of detecting a contaminant must be included in the multiple tests.

Table 2 summarises the tests applied today in order to have a complete cover of known bacteria, fungi, mycoplasma and viruses.

TABLE 2.

Test for freedom from adventitious agents

The test used to detect the possibility that a new cell substrate harbours an adventitious agent are multiple.

1. Direct observation.
2. Media for bacteria, fungi and mycoplasma.
3. Cell cultures of HEK, MK, RK, Human cell line (HeLa).
4. Laboratory animals, embryonated eggs, s/c, i/c in newborn hamsters, i/c into suckling mice, adult mice, guinea pigs and rabbits.

In addition to these tests, Stanbridge and I have been attempting to establish a test capable of rescuing incomplete viruses since these may also be a hazard. The test is based on the work of Harris in Oxford and involves the fusion of two cells which takes place in the presence of a virus (Sendai) killed by ultraviolet irradiation. The work is in its infancy, but a cell replicating T antigen and not complete virus can be induced into doing so by the fusion technique. If this test continues to show promise it will certainly become established as a means of detecting incomplete viruses in cell substrates.

A further test of the future will be fluorescent microscopy. I understand that at a recent « work shop » held at the National Institutes of Health in Bethesda this technique showed that a high percentage of monkey kidney and dog kidney tissues were shown to be contaminated.

A further test that has been suggested is examination of the cells under the electron microscope, looking for virus-like particles. Although this may be a useful test, the observations must be interpreted with extreme caution.

Heterotransplant studies.

One of the greatest difficulties in obtaining approval for a new cell substrate is the challenge made that it may carry a cancer factor. Nobody has been able to explain what this is, but it is an unanswerable criticism since many thousands of vaccines would have to be under surveillance for many years before the critics would be satisfied. In view of the known high incidence of contaminants in some cell substrates, particularly monkey kidney, a fully characterised and non-contaminated cell substrate would be an enormous step in the right direction in spite of our inability to answer the critics fully on this point.

A test that is available to us, however, is inoculation into the hamster cheek pouch to observe whether cells establish and develop into a tumour. Here again, there is not absolute correlation between the inability to establish a tumour in the cheek pouch and safety for man, but if a tumour is established then the cell substrate cannot be considered for further use.

It must be emphasised that this is a relatively insensitive test and Stanbridge and I are attempting to make laboratory animals more sensitive to the inoculation of foreign cells by treating the animals with antilymphocytic serum. Already we have obtained large tumours in mice by inoculating HeLa cells, which are rapidly rejected by an untreated mouse. Here again these tests may prove most useful in testing a new cell substrate.

Species specificity.

It seems almost superfluous to suggest that a species specificity test must be included. The most important measurement of this test is the proof that the cell population is homogeneous and has not become contaminated with another cell during the establishment of the frozen reserve of cells. There seems little to choose between the four tests available. The cell substrate must be registered with the licensing authority with results of all tests before it may be considered as a substrate for virus vaccine production.

All the tests we have been considering are those applied to a new cell substrate to establish its suitability for virus vaccine production. When it has been approved then there are additional tests on the particular batch of tissue used, to ensure freedom from contamination or abnormality, as well as tests on the virus harvest which are similar to those in force today. The advantage of using a standardised cell substrate therefore is not in reducing work but in making vaccines safer by knowing a clean substrate has been used. It is also of enormous economic importance to the manufacturer to know that the discard of a contaminated virus pool or substrate will be a rare event rather than a frequent disappointment that is being lived with today.