FAZEKAS

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Dear Joshua,

this is an urgent letter — I hope it reaches you in time, that is to say, before you get so clued-up in things influenzal that the Holy Pronouncements emanating from Canberra are taken for what they are: one-eyed and not, as you diplomatically put it, provocative.

A propos your hypothesis, here comes this week's provocation: the crucial experiments have been done, both accidentally and purposefully (Cairns, Henle) with the result that RDE treatment makes no difference to the ID/HA ratio. To me the greatest difficulty here is theoretical: if IV-formation were bound up with emergence from the last (i.e. saturating) cycle, IV should be formed whatever the starting inoculum. Actually, by the time the tissue has produced about 20% of its maximum yield, it can be shown that the tissue-bound inhibitor (this includes 1/4 of the surface RS) has disappeared. The second catch is that on your hypothesis RDE-, or for that $IKIO_4$-, treated virus should be transformable into IV in vitro, as the RS would be an integral part of the surface, and its absence or modification should render the virus non-infective, ipso facto. This is not so. To add a further catchlet, some of the strains which have the most potent enzymes (like LEE) form negligible amounts of IV on first passage in the allantois, whereas others with no enzyme action to speak of (like NWS) form IV like mad. None of these arguments is really compelling of course, but the observations will have to be reinterpreted before your hypothesis

can get off to a clean start.

Now mention here "infectivity-potentiating effect" of allantoic fluid. Put in this form the statement does not stand. What happens exactly is that deembryonated eggs yield about as much HA in synthetic medium as would whole eggs, but the ID/HA ratio is about 4. If allant. fluid is used as medium the HA yield is about 100-fold down, but the ID/HA ratio is back to normal (~ 6.2). This could be due to prevention of infection by the original seed; however the eggs dont yield more even after 48 hrs. It could be due to masking of the yielded HA, but not of infectivity; however, RDE does not release any virus, the preps dont bind more antibody than expected from their apparent virus titre, they have no greater enzyme activity or interfering power. For these reasons I think less virus is produced under these conditions, but what comes out of the cells is infective. I have no clue why the yield is so low. The phenomenon is shown by allantoic fluid (native, dialysed or heated) but <u>not</u> by all. fl. treated with $IdIO_4$ or diluted 1:5; it is almost independent of virus dose. The latter tend to exclude any masking effect as $IdIO_4$-treated inhibitor is the best masker of flu virus I know, and what it does here is exactly the reverse. Further, the eggs do not yield more even if they are thoroughly washed at 4 hrs, and the yield collected into saline. Conversely, those infected in saline and filled up at 4 hrs with allant. fluid yield high-titre HA, 99% of which is non-infective. This is the main reason for my linking IV production with entry rather than exit from the cells.

~~As regards the~~

Your third letter arrived just this moment — it catches me in the rather unpainly pose of one trying to teach his grandmother to suck eggs. So without any further generation of hot air I'll answer your questions as best I can.

Membrane inhibitor. I believe, with Cairns, that most of the inhibitor comes from behind the endothelial layer, and that RDE in vivo destroys only extracellular (surface) substrate. I do not agree with Schlesinger + Karzon when they say heated virus is bound by RDE-d cells. This is an expt one does almost daily, and I think everybody else finds the opposite. I don't have their paper before me, but if I remember correctly it happened only in the whole allantois where heated virus is irreversibly bound to the all. fl. inhibitor after a long time. This is the same phenomenon Burnet has shown for indicator virus and RBC, and Horsfall very clearly with allantoic fluid. Neither is Schlesinger the first who fell into this trap.

What I find most puzzling about the inhibitor is that adsorbed virus, dead or alive, can be removed from the membrane before grinding, but not after. Emerging virus, presumably adsorbed to the same surface, is removed both before and after grinding. This happens even after subsaturating inocula where most of the cells had no experience of the virus. (I think some sort of vascency has to be considered because of such differences between first and later cycles.)

Incompleteness as natural intracellular state. I think there is no observation in favour of virus existing inside the cell, either as infective or non-infective particle. Cairns has looked into this probably more carefully than anybody else, and thinks that what is usually called intracellular is virus trapped between cells. As it is a constant fraction of fluid-virus, it can't be its precursor but must be one side,

of a partition equilibrium. I think this fraction does not participate in multiplication, and this is my evidence for it: If the allantois is exposed to high concentrations of antibody between 4 and 12 hrs after infection with, say, 10^4 ID_{50}, there is no further multiplication of virus (or, occasionally, as if it started from ~10 particles at 12 hrs), and the tissue is infectible by a new dose of virus. The same tissue yields ~ 10^3 ID_{50} after grinding. If the antibody treatment is done intermittently, i.e. 4 two-hour spells with one hour's rest between them, multiplication continues throughout, although at a reduced rate, and at 15 hrs is goes on as if there were ~10^6 ID_{50} present. This argues against lateral spread which Cairns had to postulate to account for Henle's very prolonged release of virus. Henle's finding of lower ID/HA ratios for tissue-bound virus (barely significant, as I remember) might be due to this virus having no turnover and therefore is exposed to heat-inactivation over the whole span of its existence, while the fluid-virus flits from cell to cell and spends only a short time in the fluid after emergence. However, Henle hardly ever says something that is not well established, and he believes quite firmly that IV is an intermediate in virus production.

<u>Multiplicity of infection needed for IV production</u>. I had a letter from Cairns a few days ago: apparently in the August issue of Virology Henle & Ledinko have repeated the Cairns & Edney expt in tissue culture, with the same general results. He does not say anything about multiplicity, but it is quite clear that the party line has veered from IV-production at $m < 1$, and this point has not been a strong one ever since it was found that there are 10 visible particles for each infective unit, and that there are 2.5 times less cells than we thought in 1950.

Yet, to show some rearguard activity, the concept of multiplicity still implies diminishing ID/HA ratios as m increases [contrary to evidence], or the unpalatable assumption that one and only one infective particle is yielded by each multiply infected cell, irrespective of multiplicity. This latter concept becomes very awkward indeed when one starts thinking about continued-passage data.

Allantoic fluid effect. The main results are in yesterday's part of the letter. Technique: 14-d eggs emptied from the sharp end; 2.5 ml basic medium (either 14-d all. fluid or SM) + 0.25 ml fresh HCT, titre 10 by Hall Inst. method; 16-20 hrs rolling at 36°C; later HA & inf. titrated immediately. I have done the basic expt (with various frills) three times. As I told you, it was at this time that our new students arrived, and the two large tests I did last November are still sitting at -70° waiting to be titrated. The switching of the media at 4 hrs is, to my mind, the crucial test although probably not quite enough to sway the helix-happy minds of the Hall Institute.

Effect of KIO_4 + glycerol. Of course I have done this as a control, and have repeated and expanded the tests on seeing Henle's paper. In my hands the IO_4^--glycerol mixture, or IO_3^-, or the equivalent amount of formaldehyde, or a mixture of CH_2O and IO_3^- has no effect. Further, the IO_4-effect is unchanged when the eggs are washed after infection. The rate of inactivation Hin + al find is insufficient to account for the results, especially when HCT harvested within the hour of leaving the cells. In the case of other strains which produce IV anyway, the effect of KIO_4 should be additive as it would be considered as superimposed inactivation,

This is not so. Also, the phenomenon is bound to the presence of allantoic fluid: virus made up in saline is not affected by KIO_4. However, I don't think irreversible combination with inhibitor has much to do with it; partly because there isn't enough inhibitor in these fluids to begin with, and mainly because the effect could never be a decrease in the ID/HA ratio, as there is no known inhibitor which would be more effective against infectivity than against HA. (From all this you may derive the tentative conclusion that I seem to stick to my guns.)

<u>Production of NIHA on superinfection</u>. (This probably caused another day's delay in sending off this letter as we had to get the volume of JEM back from the binders.) I don't think there is much in this expt, and the conclusions a certainly not justified. The viropexis rate of this strain is, conservatively, about 2 hrs extracellular half-life. Hence, at 2 hrs $10^{5.7}$ out of 10^6 ID_{50} have been taken up of the first inoculum; this occupies about 2% of the available cells. Then comes the massive dose of $10^{9.6}$ $UP_{(H)}$, i.e. multiplicity ~ 100, and within three minutes > $10^{7.8}$ of this will be within the cells thus establishing a multiplicity 50x greater than that of the first inoculum at this time. Within half-an-hour the multiplicity of even the infective fraction has caught up with that of the 1st inoculum, as the input was 4x higher ($10^{6.6}$). If one waits for 6 hrs or more, the original inoculum has multiplied by a factor of ~ 100, has become "nascent" (i.e. gets into the next cell at about ten times its pre-first-cycle rate) and will stand up to all comers, especially as far as infectivity is concerned. This is, I think,

what the results show: within the first cycle $UP_{(+)}$ wins on numbers; after the first cycle quality tells, and SP can do it with one linkage group tied behind its back.

Bit technique: Dear Sir/Madam,

Our Travelling Salesman will call on you Thursday, 26th inst., to demonstrate the abovementioned articles. Any complaints should be addressed directly to the Management.

In anticipation of your custom,

We remain, etc.

Extra item: I have no firm plans for the second half of next year, as all depends on whether Canberra's come good or not. If all goes well I should reach Madison towards the end of November, and should be able to shift it by ±3 weeks if necessary. If all does not go well I shall have to restrict my stay to the 14th December from 14^{00} to 14^{15}, and content myself with inquiring about the Wisconsin weather from the airport attendant whose duty it is to de-louse overseas passengers on touching down.

That, I think, is all. I feel I should apologize for the prolixity (coupled with an illegible hand) — but this is one of the common ploys to be used when one is not at all sure of what's going on. What's worse, it gives me the itch to start up again on IV which, under the circumstances, is a bad thing.

Yours,

Stephen.