November 16, 1949

What I am going to say tonight is based on experiments which Dr. Aaron Novick and I carried out between April and June of this year.

You may find a detailed account of these experiments in the October issue of The Proceedings of the National Academy.

When Dr. Kelner published his discovery of light reactivation we did not have any intention to make an extended investigation of this phenomenon.

But we thought it would be nice to see, if we could find the effect which he described.

So we thought we would just take an afternoon off and see if we could get the effect with a strain of coli which we had on hand.

This happened to be the same strain B/r which Dr. Kelner used in the experiments about which he told us tonight.

But while we used the same strain as did Dr. Kelner, we treated our cultures differently.

Our bacteria were taken from the exponential growth phase; they were then transferred into saline and kept in saline at 37° for about 16 hours before we exposed them to ultraviolet irradiation

Fig. 1

Fig. 1 shows the survival curve obtained with such cultures in a semi-logarithmic plot.

You see that the number of survivors falls at first very slowly with increasing U-V dose; then it falls faster, and finally the survivor curve goes over into a straight line.

Starting with 10^8 bacteria, the straight line portion of the survivor curve extrapolates back to zero dose to about 10^{14} bacteria.

This extrapolated value characterizes the shape of our survivor curve and it does not depend on what units we use for plotting the U-V dose.

The curve B shows the survivor curve which we obtain if we follow the U-V irradiation by exposing the bacteria to the light of a 1,000 watt projection lamp at a distance of 8" for about one hour.

The two survivor curves, A and B, have the same shape. And we can see that by extrapolating back the straight line portion of the survivor curve B to zero dose we get back to the same point of about 10^4 bacteria which we obtained before for the curve A.

For every U-V dose D for which we obtain a given number of survivors with light reactivation (according to the curve B) there can be found a lower U-V dose L which would give the same number of survivors without light reactivation, (according to the curve A.) [show] Thus

From the two survivor curves A and B we can determine for every dose D the corresponding dose L and if we plot L as a function of D we obtain the straight line shown in Fig. 1. (LIGHT)

This means that we may write

$$B(D) = A(L)\,;\; L = qD\,;\; B(D) = A(qD) \sim \text{No 1}$$

$q = 0.4$

We can express this result by saying the following: If we expose the bacteria to an U-V irradiation and then shine strong light on them for about an hour, the effect of the light on the number of the survivors consists in reducing the effective U-V dose by factor q and this factor q is independent of the U-V dose. In the series of experiments shown in Fig 1 we had $q = 0.4$

At this point it seems useful to make certain assumptions for the sake of argument.

These assumptions might have no final validity and they must be taken with more than just one grain of salt.

But they do permit me to give you a very simple presentation of all the experimental results which we have obtained thus far.

These assumptions are as follows:

1. That an U-V dose produces in the bacteria a "poison" and that it produces this poison in an amount which is proportionate to the dose D. "Poison" = D
2. That this poison is present in two forms: a form P_x which is present in an amount x_0 and which is not sensitive to light;

and another form P_y which is present in an amount of y_o and which is destroyed by light.

We have then $x_o + y_o = D$

3. That the ratio of the amounts of these two forms of the poison x/y is independent of the U-V dose D.

4. That if bacteria are exposed to U-V and subsequently are reactivated to a lesser or greater degree by light, the number of survivors is determined by the (label) amount of poison $x + y$ which is left in the bacteria after light reactivation.

$B = f(x + y)$

On the basis of these assumptions we can now interpret the relationship

$$B(D) = A(qD) \qquad \text{No 1}$$

by saying the following: if we take bacteria which have been exposed to an U-V irradiation, and if we shine strong light on them for one or two hours, we destroy all the poison that is in the form P_y (light sensitive) and we are left only with the poison which was (originally) present in the form of P_x and which (accordingly) was present in the amount x_o.

If we then write

$$x_o = qD$$

where q is a constant that is independent of the U-V dose, then it follows that we must have

$$B(D) = A(qD) \qquad \text{No 1}$$

We can now go one step further and make the assumption that the light sensitive variety of the poison P_y, which is destroyed by light, is destroyed by light at a rate which is at any time proportionate to the amount in which this poison is present at that time.

On the basis of this assumption we may write :

$$\frac{dy}{dt} = -\alpha y \qquad \text{No 2}$$

Here y is the amount of the ~~poison P~~ light sensitive vacuoles of the poison present at the time t and α is a constant that must be independent of the U-V dose D.

α will of course depend on the light intensity and it can be expected to increase with increasing light intensity.

This equation permits us to predict the number of survivors, which we shall obtain if we first expose the bacteria to a given U-V dose and ~~xhaxxexpxxxxxhxx~~ subsequently expose these bacteria for varying lengths of time to light of a given intensity.

A convenient way of expressing the number of survivors is to express this number in terms of the corresponding L value. That is, we may express the number of survivors in terms of the U-V dose L which would give the same number of survivors ~~xcxcxdingxcxxthe xurvivexxcxcxexA~~ in the absence of light reactivation, (according to the curve A.)

Once we have the survivor curve A for the particular culture which we use, we can very easily read from the curve for each value of the survivors, which we find in any particular experiment, the corresponding L value.

Expressed in terms of these L values, it follows from the equation, which we have postulated, that the number of survivors will be given by the following expressions:

$$L = x_0 + (D - x_0)\, e^{-\alpha t} \qquad \text{or} \qquad \ln(L - L_\infty) = \ln(D - L_\infty) - \alpha t$$

In these expressions L is the L value that corresponds to the number of survivors which we ~~xhaxx~~ find if we permit light reactivation to proceed for a period of time t.

The symbol L_∞ stands for the L value which one obtains after a very long exposure to light; an exposure which is so long that it gives us the maximum amount of light reactivation that is obtainable.

As you see from the second of these formulae we are to obtain a straight line if we plot $\ln(L - L_\infty)$ as a function of time. The slope of that straight line should give us the value of the constant α.

These expressions give us the value of L as a function of t, where t is the period of time during which the bacteria are exposed to the reactivating light.

The symbol L_∞ stands for the L value which we obtain after a very long exposure to light; an exposure which is so long that it gives us the maximum amount of light-reactivation that is obtainable.

As you see from the second of these formulae (No. 3) we ought to obtain a straight line if we plot $\ln(L - L_\infty)$ as a function of time. And the slope of that straight line should give us the value of the constant k.

Fig. 2

Fig. 2 shows the experimental results.

In this figure you see two straight lines which correspond to two different doses of U-V exposure.

You see that the slope of both of these lines is the same, which means that the constant α is independent of the U-V dose as it should be.

You may see, however, also from this figure that these two straight lines do not extrapolate back for zero time of light reactivation to the L values which we have experimentally obtained for $t=0$ that is in the absence of light reactivation.

This discrepancy can be quantitatively expressed by saying that there is a latent period τ for light-reactivation which in these particular experiments had a value of about three minutes. (LIGHT)

In these particular experiments we used for light reactivation a 1,000 watt lamp at 8" from the bacterial culture.

If we use weaker light we obtain higher values for the latent period τ and we also obtain lower values for the slope of the straight lines, that is, we obtain lower values for the constant α.

Fig. 3

In Fig. 3 shows that if we plot the L values as a function of the UV dose, not for full light reactivation, but for light reactivation which is carried out for shorter periods of time, namely, 20 minutes, 25 minutes, and 30 minutes, we again obtain straight lines as we did for full light reactivation as shown plotted in Fig. 1.

This is what we should expect, assuming that both the constant α and the latent period τ are independent of the U-V dose D.

(LIGHT)

You see, then, that the very simple assumptions which we made are in good agreement with all the experimental data that we have so far obtained.

It would be a mistake, of course, to conclude that these assumptions are necessarily correct.

Let us, however, for a little while longer stick to these assumptions.

We now ask ourselves the following questions:

U-V exposure of coli leads not only to the killing of bacteria, but it also produces mutations.

Does the poison which we assume to be responsible for the killing of bacteria, also determine the number of mutants which we find among the progeny of the bacteria that have been exposed to U-V irradiation?

In order to answer this question, we made experiments in which we used three different mutants of our coli strain.

We used mutants resistant to one of the bacterial viruses T_4 or T_6 or T_1.

If we expose bacteria to a U-V dose and then allow the bacteria to grow in liquid culture, we can determine the relative abundance of such mutants in the culture as a function of the number of generations through which we allow the culture to go.

The next figure shows the type of curve which we obtain in such experiments.

Fig. 4

In Fig. 4 we have plotted the relative abundance of mutants resistant to the bacterial virus T_1, as a function of the number of generations through which the bacteria went in liquid culture.

The relative abundance of these mutants rises with the number of generations, first slowly, then faster, and finally it levels off when the time the bacteria have gone through about ten generations.

Beyond that there will be a slow decrease in the relative abundance of the mutants which should continue until the level of the natural equilibrium is reached.

(Wright)

It follows, that if we wish to study the effect of U-V irradiation and light-reactivation on the relative abundance of mutants among the progeny of the bacteria, we might obtain significant results if we follow this recipe: After irradiation of the bacteria we let them grow in liquid culture for ten generations and then determine the relative abundance of the mutants.

The next figure shows the experimental results which were obtained by following this recipe

Fig 5

In Fig. 5 we have plotted the relative abundance of mutants as a function of the U- V dose for three different mutants, each of them resistant to one of the three bacterial viruses T_4, T_6, or T_1.

The results are shown in this figure in a loglog plot.

When you plot something on a loglog scale you can be almost certain to get a straight line and it is therefore not very surprising that all curves in this plot are straight lines.

However, all these straight lines appear to be parallel to each other and this might perhaps have some significance.

The lines marked with a capital M give the relative abundance of the mutants obtained in the absence of light reactivation, and the lines marked with a capital N give the relative abundance of the mutants obtained with full light reactivation.

You see that for any given U-V dose, the relative abundance of the mutants is lower for the light reactivated cultures.

The straight lines N obtained with light reactivation are each displaced by about the same interval with respect to the corresponding straight line M obtained without light reactivation in the dark.

If we designate the mutants obtained in the dark with M_4, M_6, M_1 and the mutants obtained with light reactivation with N_4, N_6, N_1 we may write on the basis of the curves obtained

$$N_4(D) = M_4(q_4 D)$$

For He and this may be compared with the survivor curves A and B obtained in this same series of experiments for which we found

$$B(D) = A(qD) \qquad q = 0.35$$

Within our experimental error we have

$m_4 = m_6 = m_1 = qf$

It seems therefore that the effect of light reactivation on the appearance of mutants, among the progeny of the U-V irradiated bacteria, is the same as the effect of light reactivation on the number of the survivors.

In both cases the effect of light reactivation appears to consist in the reduction of the effective U-V dose, by the same factor q.

This factor q is not dependent on the U-V dose but its value seems to depend slightly on the way in which the culture is prepared.

Therefore, within the limits of our experimental error we may write

$$m_4 = m_6 = m_1 = q$$

It seems therefore that the effect of light reactivation on the appearance of mutants, among the progeny of the U-V irradiated bacteria, is the same as the effect of light reactivation on the number of survivors.

In both cases the effect of light reactivation appears to consist in the reduction of the effective U-V dose, by the same factor q, which is not dependent on the U-V dose and which in this last series of experiments had a value of .35.

I have to add one word of caution, and I have to emphasize that we have not investigated the U-V induced mutations with the same thoroughness, as we have investigated the effect of U-V exposure on the number of survivors.

I also have to emphasize that the assumption, that we have to deal here with a poison, was made merely for the sake of permitting a simple presentation of our experimental results.

We have no chemical evidence in our experiments that would permit us to choose between a theory, that assumes that killing and mutation are caused by poison, and the so-called hit theory, that assumes a more direct biological effect of irradiation.

You may imagine, for instance, that this is what happens in our experiments: The U-V irradiation changes a certain type of chemical bonds in a number of places in the genetic material contained in our bacteria.

According to this view the U-V irradiation causes lesions in the genetic material and these lesions are of two kinds; one kind which can be healed by light, and another kind which cannot be healed by light.

Depending upon the number of lesions, which remain unhealed after light reactivation, we obtain a certain number of survivors and a certain relative abundance of mutants.

A hit theory of this type fits in just as well with the results so far obtained by us, as would a theory which assumes the production of a poison in the conventional sense of the term.

In order to decide between the two theories, we must ask ourselves whether we can interpret the killing of the bacteria (observed in our experiments) as lethal mutations, and whether we can understand on this basis, the shape of the survivor curves.

This is a point which we are investigating at present.

Fig. 6

In Fig. 6 you see two survivor curves, one of the curves relates to bacteria which were exposed to different U-V doses, while the culture was in the exponential growth phase.

The other survivor curve relates to bacteria which had been kept in saline at 37° for 16 hours.

You see, that the shapes of these two curves are very different but the straight line portions of the two curves appear to be parallel.

This observation seems to point in the direction of the hit theory. At least it gives us a clue which we intend to follow up.

Starting from this point, we intend to study the survival and the mutations observed following U-V exposure in cultures which differ in the shape of the survivor curve.

Whether we shall get anywhere with this approach, we do not know.

But in any case it seems to be more advisable to do the experiments first and to talk about them afterwards, rather than the other way round -- and this being so, I have reached the end of my talk.