The second process is the inactivation of provirus[4] and fits the curve $1-(1-e^{-s_3 D})^m$,[6] where s_3 is the cross-section for proviral inactivation and m is the multiplicity of hit. It is important to note that this multi-hit curve does not necessarily indicate that there is more than one provirus. Not enough data has been collected as yet to determine the wavelength dependence of s_3. In summary the overall dose effect curve is $N = N_0(1-e^{-s_1 D})\,[1-(1-e^{-s_3 D})^m]$. Fortunately the two portions of the curve can be analyzed separately to a fair approximation (cf. Fig. 1).

The graph of the logarithm of bacterial survivors versus dose also gives a straight line owing to the direct inactivation of haploid K-12 cells by a single "hit" process plus the disappearance of cells due to lysis, i.e. $n = n_0 e^{-(s_1+s_2)D}$, where n is the number of bacterial survivors and s_2 is the cross-section for cell inactivation (Fig. 1).

E. coli K-12 $Lp_1^+ Lp_2^s$ [7] was grown in a peptone medium to a titer of $2 \cdot 10^9$ cells/ml, washed twice in phosphate buffer (pH 6.9) and diluted by a factor of 10^4 before irradiation with monochromatic light of known intensity. At various times aliquots were diluted in broth and incubated with shaking at 37° C for 45 minutes. Platings were then made according to the techniques of WEIGLE AND DELBRUCK[4]. The cell absorption shown (Fig. 2) is that of a titer of $8.1 \cdot 10^8$ cells/ml harvested from peptone broth in the log phase and resuspended in buffer (pH 6.9) after four washings, and is corrected for scattering.

The induction spectrum has a sharp peak at 265 mμ, falling off rapidly on either side, whereas the cell inactivation peak is broader with a slight shoulder at 280 mμ (Fig. 2). Thus both curves indicate nucleic acid chromophores, while the cell inactivation curve may indicate some protein involvement also (cf. LOOFBOUROW[8]). Neither action spectrum follows cell absorption. The rapid drop in cross-section below 240 mμ, with virtually no cell inactivation at 237.5 mμ, indicates heavy absorption in peripheral regions which are not involved with induction or cell inactivation. The fact that more energy is needed to induce than inactivate may indicate that the inactivated cells can still support phage growth. This has already been demonstrated for *E. coli* C invaded by λ phage[9]. It also shows that the inducible substance is not identical with all the units concerned with cell inactivation. Thus induction may involve the activation or inactivation of a particular enzyme forming system, or the direct activation of provirus.

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THE OCCURRENCE OF FREE THIAMINE PYROPHOSPHATE IN THE SOLUBLE FRACTION OF RAT LIVER HOMOGENATE*

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In a previous note[1] it was reported that centrifugally prepared mitochondrial and soluble fractions of rat-liver homogenate[2] contained practically equal proportions of the cellular thiamine pyrophosphate (TPP). The nuclear fractions and the microsomes were essentially free of TPP. The virtual absence of TPP from rat-liver nuclei was confirmed by applying a recently published method

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for the isolation of cell nuclei[3] which proved to be very satisfactory from a morphological point of view[4]. The same pattern of distribution was found for mouse liver and subcutaneously transplanted mouse hepatoma, though the overall content of TPP was much lower in the latter tissue[4].

As pointed out previously[1] there is ample reason to assume that the large percentage of TPP contained in the soluble fraction has not been artificially released from the mitochondria. Because free TPP is apt to be broken down by tissue phosphatases[5], it was considered likely that the TPP of the soluble fraction would be bound to protein. The present investigation was performed in order to obtain confirmative evidence.

Soluble fraction was prepared by centrifuging a homogenate of 1 part of normal rat liver in 4 parts 0.25 *M* sucrose solution at 15,000 × *g* for 30 minutes. The occurrence of microsomes, possibly even the majority of them in such preparations, could not, in our opinion, affect our results.

Indirect information as to the state of the "soluble" TPP was obtained by the following experiment. A portion of the supernatant was boiled at pH 3 in order to liberate TPP bound to protein, and then brought to pH 6.2. Another portion was brought to pH 6.2 without previous boiling. 1 ml samples of both portions were then transferred to Warburg vessels together with alkaline washed yeast and pyruvate under the conditions used for the determination of TPP by the carboxylase method[6]. It can be safely assumed that by this method only free TPP is estimated. In our experiment 4 rats were used. The measured contents of TPP in γ per ml soluble fraction were 1.2, 1.3, 1.3 and 1.2 in case of the boiled supernatant and 1.3, 1.3, 1.6 and 1.5 in case of the original supernatant. This result is strongly suggestive of the occurrence of TPP in free condition in the cellular fluid.

As a more direct test for the condition of the "soluble" TPP a sample of soluble fraction was passed at + 4° C through a protein-tight collodion filter. As the gradual increase in colloid osmotic pressure of the residue reduced the filtration rate continuously, the operation was terminated after 1 hour when only 1.6 to 1.8 ml of the fluid had passed. The contents of TPP were determined in both the ultrafiltrate and the original soluble fraction which was kept at the same temperature. The results (Table I) show that, despite the fact that all protein was withheld by the filter membrane, the larger proportion of the TPP had passed the filter. The loss of TPP may be due to adsorption at the membrane or to increased decomposition in the thick protein layer at the filter surface.

TABLE I

AMOUNTS OF THIAMINE PYROPHOSPHATE IN THE SOLUBLE FRACTION OF RAT LIVER BEFORE AND AFTER ULTRAFILTRATION

Rat No.	*γ TPP per ml*	
	Original soluble fraction	*Ultrafiltrate*
1	1.8	1.1
2	2.1	1.9
3	1.8	1.1

These results thus also show that a large percentage of the cellular TPP occurs in the cytoplasmic fluid in free condition. It seems plausible to presume that within the living cell this form of TPP, though liable to decomposition by phosphatase, is resynthesized at an equal rate by rephosphorylation of the formed thiamine[5,7].

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators.

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