investigated, and conditions for the estimation of the enzyme have been established.

2. In 0.05M citrate buffer, the enzyme shows optimum activity at pH 4.3. The optimum substrate concentration cannot be attained owing to the limited solubility of the substrate.

3. *N*-Acetyl- β -glucosaminidase is inhibited competitively by *N*-acetylglucosamine, acetamide and acetate.

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The Action of 2:4-Dinitrophenol on Myosin and Mitochondrial Adenosine Triphosphatase Systems

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Mitochondria freshly isolated from liver show low or negligible adenosine triphosphatase (ATPase) activity towards added substrate, but on addition of low concentrations of 2:4-dinitrophenol (DNP), adenosine triphosphate (ATP) is hydrolysed at a rapid rate (Hunter, 1951; cf. Lardy, 1945). Similar, though perhaps less striking, effects have been obtained with mitochondria isolated from skeletal muscle (Chappell & Perry, 1954). The precise nature of this effect of DNP is not understood, although, significantly, it is accompanied by the loss of oxidative phosphorylating activity (Loomis & Lipmann, 1948; Cross, Taggart, Covo & Green, 1949) and by the inability of ATP to reverse the swelling induced in mitochondria of pigeon breast muscle by hypotonic media (Chappell & Perry, 1954). In isolated mitochondria the action of DNP is complex and may involve, amongst other things, effects on the permeability of the membrane; but it appears from the work of Lardy & Wellman (1953) on the ATPase activity of aqueous extracts of mitochondrial acetone-dried powders that DNP has a specific stimulatory effect on the ATPase enzyme itself. Furthermore, the observation that DNP stimulates the ATPase activity of myosin (Webster, 1953) suggests that DNP can have an effect on the mechanism of ATP hydrolysis when it is catalysed by other enzymic systems.

In previous studies on the metabolism of ATP by intracellular components of muscle (Chappell, 1954; Chappell & Perry, 1953, 1954) we found that

under the ionic conditions in which DNP stimulated the enzymic activity of L-myosin (Weber & Portzehl, 1952), 0.1–5 mM DNP failed to stimulate the myofibrillar ATPase activity of rabbit myofibrils. The present communication is concerned with a general investigation of the effects of DNP on the ATPase activity both of myosin and isolated myofibrils and shows that the presence of actin modifies the action of DNP on the enzymic activity of myosin. The response of the latter system to DNP has been compared with that of the mitochondrial ATPase.

Certain aspects of this work have already been reported (Chappell & Perry, 1955). In an associated communication, Greville & Needham (1955) reported similar findings on the DNP stimulation of L-myosin ATPase activity.

METHODS

Myofibrils. Myofibrils were prepared in a medium containing 0.039M borate buffer, pH 7.1, and 0.025M-KCl (borate-KCl) as previously described (Perry & Grey, 1956). Stock preparations were stored at 0°, and for enzyme experiments they were used within 10–12 days, diluted with borate-KCl to give suspensions containing 5–7 mg. of protein/ml.

Myofibrillar proteins. L-Myosin was prepared by a method described elsewhere (Perry, 1955) which combines the experience of a number of workers. The method of Guba & Szent-Györgyi (1945) was used for the preparation of G-actin solutions. G-Actin was purified by isoelectric

precipitation with 0.02M sodium acetate buffer, pH 4.8. The concentrated F-actin solution (10–20 mg. of protein/ml.) which was obtained on restoring the pH to 7 was stored at 0°.

Mitochondria and mitochondrial extracts. Rat-liver mitochondria were prepared in 0.25M sucrose as described by Schneider (1948), and pigeon breast-muscle mitochondria by the method of Chappell & Perry (1954). The method of Lardy & Wellman (1953) was employed for the preparation of acetone-dried powders of rat-liver mitochondria. To obtain extracts these powders were homogenized at 0° with 20 vol. of glass-distilled water in a pestle-type homogenizer, allowed to stand 30 min. at 0° and centrifuged for 20 min. at 1000 g. The supernatant was used for ATPase experiments.

ATPase experiments. Enzyme experiments were carried out in general as described earlier (Perry & Grey, 1956), at 0° or 20°. The reagents were pre-incubated for 5–15 min. at the incubation temperature and the reaction was started by addition of 0.2 ml. of enzyme suspension. Unless otherwise stated, the ATP (sodium salt, obtained from Sigma Chemical Co., St Louis, U.S.A.) was used at a concentration of

0.005M. The pH values of buffer solutions were in all cases measured at 20°.

2:4-Dinitrophenol. This was twice recrystallized either from ethanol or water.

RESULTS

Effect of DNP on the calcium-activated ATPase of myofibrils and L-myosin. The addition of 1–10 mM DNP to the standard incubation medium produced a progressive inhibition of the calcium-activated ATPase (Ca-ATPase) of myofibrils prepared in borate-KCl. In the presence of 0.05M-KCl, a similar range of DNP concentrations produced little change in ATPase activity, but as the ionic strength of the medium was increased still further a marked stimulation of the Ca-ATPase activity by DNP was obtained (Fig. 1a). Although the addition of the higher concentrations of KCl decreased the ATPase activity in the absence of DNP, the maximum

Fig. 1. Effect of DNP on the Ca-ATPase of (a) myofibrils (3 days old) and (b) L-myosin, at varying ionic strengths. Incubations were carried out for 10 min. at 20° in a total volume of 2 ml. containing 5 mM ATP, 5 mM-CaCl₂, 0.05M tris buffer (pH 7.4), 0.1 ml. of myofibrils in borate-KCl or myosin (about 0.06 mg. of N), in 0.04M-KCl, and the following further additions: ○, nil; ■, 0.05M-KCl; □, 0.1M-KCl; △, 0.2M-KCl; ●, 0.4M-KCl. Activity in the absence of added DNP and KCl was taken as 100.

stimulation obtained in the presence of the phenol was greater than the depression of activity produced by salt alone.

The Ca-ATPase of L-myosin was also markedly stimulated by concentrations of DNP similar to those used in the experiments with myofibrils (Fig. 1*b*). A major difference existed in the response of myosin to DNP, however, in that stimulation was achieved in the absence of added KCl, i.e. at the ionic strength of the incubation medium (approx. 0.06).

The effect of KCl on the Ca-ATPase of the myofibrillar system was not a specific one but rather an effect of ionic strength, for NaCl was equally effective and for a given DNP concentration K_2SO_4 produced maximal stimulation at a lower concentration.

In the myofibril the myosin is associated with actin and it seemed probable that the presence of the latter protein was responsible for the difference in response to DNP of the ATPases of the two systems. The fact that another property of myosin which is modified by actin, i.e. magnesium activation of the ATPase, is likewise sensitive to ionic strength over a similar range, supported this view. To check this hypothesis, synthetic actomyosins containing a fixed amount of myosin and varying amounts of actin were prepared and the effect of DNP on their ATPase activities in the standard incubation medium (*I* approx. 0.06) was determined. The results of one such experiment are

Fig. 2. Effect of actin on the stimulation of the Ca-ATPase of myosin by DNP. Incubation medium contained 0.05M tris buffer (pH 7.4), 5 mM- $CaCl_2$, 5 mM ATP and 0.2 ml. of enzyme suspension in 0.05M-KCl; final volume, 2 ml. The enzyme solutions contained a fixed amount of myosin (0.28 mg. of N/ml.). ○, No further additions; □, 5 mM DNP; ●, 0.2M-KCl; ■, 0.2M-KCl + 5 mM DNP.

illustrated in Fig. 2 and indicate that when the ratio myosin N:actin N was 2, DNP no longer stimulated the ATPase activity. They also show that the stimulating effect of DNP is restored by the addition of 0.2M-KCl to this actomyosin system.

Effect of DNP on the magnesium-activated myofibrillar ATPase. When magnesium was used to activate the myofibrillar ATPase (Mg-ATPase), an increase in the ionic strength progressively decreased the activation effect, which was entirely absent when the incubation medium contained 0.2M-KCl. Nevertheless, with freshly prepared myofibrils, magnesium appreciably stimulated the ATPase in the presence of 0.1M-KCl (see Perry & Grey, 1956). Addition of DNP to this system at concentrations of 0.1 and 1 mM had little or no effect on the Mg-ATPase; 5 mM DNP inhibited at all levels of ionic strength (Fig. 3). This is in contrast with the effect on Ca-ATPase, where, under similar ionic conditions, stimulation by DNP increased progressively with concentration up to a maximum at 8 mM.

Effect of DNP on manganese-activated myofibrillar ATPase. Activation of the myofibrillar ATPase by $MnCl_2$ resembled the effects obtained with $MgCl_2$ in that with increasing ionic strength the activation fell off sharply until, in the presence of 0.2M-KCl, the ATPase activity was at a low level. The addition of increasing amounts of DNP, in the presence of KCl up to 0.05M, produced inhibition or activation,

Fig. 3. Effect of DNP on the Mg-ATPase of myofibrils at varying KCl concentrations. Myofibrils, 1 day old. Incubations were carried out for 5 min. at 20° with 5 mM ATP, 5 mM- $MgCl_2$, 0.05M tris buffer (pH 7.4), and the following further additions: ○, nil; □, 0.05M-KCl; ●, 0.1M-KCl; ■, 0.2M-KCl.

neither of which was very pronounced. At higher KCl concentrations the ATPase activity was lower, but increasing amounts of DNP produced stimulation. Thus, although at higher ionic strengths greater ATPase activity was obtained with calcium than with manganese, the systems were similar in that activity in both cases was stimulated by DNP.

Reversibility of DNP effect. A number of experiments were performed which demonstrated that after the myofibrillar Ca-ATPase had been stimulated by DNP under typical conditions, and the phenol largely removed by dialysis, restimulation occurred on addition of fresh DNP. Samples of both control and DNP-treated myofibrils were dialysed against the borate-KCl medium and then assayed for the DNP effect in the presence and absence of 0.2M-KCl. The results of a typical experiment are presented in Table 1.

An attempt was made to assay the amount of ATPase brought into solution under the conditions used to study stimulation of the Ca-ATPase by DNP. This was done by cooling to 0° a sample of the complete incubation mixture after incubation with and without DNP, and then centrifuging at this temperature for 5 min. at 20 000 g. The supernatant, which was considered to contain any soluble ATPase, was then assayed again at 20° in a medium identical with the original. In the absence of added KCl no Ca-ATPase activity passed into solution, but with added KCl (0.2M) most of the ATPase-active material activity was rendered soluble. In general the results indicated that, under conditions in which DNP stimulated the myofibrillar ATPase, the enzyme was partly in solution. Nevertheless, DNP did not invariably stimulate when the enzyme was in solution, for although 60–70 % of the ATPase-active material was soluble in the presence of 0.2M-KCl at 0°, DNP produced inhibition under these conditions.

Effect of DNP on calcium-activated ATPase at 0°. When the effect of DNP on the Ca-ATPase of L-myosin and myofibrils was compared at 0° rather than at 20° it was noted that many of the differences observed in the behaviour of the two systems at the higher temperature had disappeared. For example, at 0° in the absence of added KCl, increasing DNP concentration caused progressive inhibition of the Ca-ATPase of L-myosin, and stimulation was not obtained until the concn. of KCl was 0.4M (Fig. 4b). Similar results were obtained with the Ca-ATPase of the myofibrils (Fig. 4a), and apart from the fact that in the absence of DNP increasing ionic strength caused a greater increase in enzymic activity with myosin than with myofibrils, the sets of curves obtained were almost identical.

It was noted that on cooling to 0° the standard incubation medium containing 0.05M aminotris-hydroxymethylmethane (tris) buffer, pH 7.4, at 20°, the pH rose by approximately 0.4. This change in pH of the medium on cooling, however, was not responsible for the different behaviour of DNP at 0°, for the following reasons. At 20° no significant difference could be detected in the behaviour of L-myosin to DNP in 0.05M tris, pH 7.4 or pH 7.8. Likewise the behaviour of L-myosin in 0.05M glyoxaline buffer, pH 7.0, to DNP at 0° and 20° was very similar to that observed under similar conditions in 0.05M tris, pH 7.4.

Magnesium activation of the myofibrillar ATPase at 0°. The result obtained on reducing the temperature from 20° to 0° for the DNP stimulation of the Ca-ATPase of myosin prompted the study of certain other properties of the enzyme at the lower temperature. In particular it was noted that at 0° magnesium inhibited the myofibrillar ATPase at approximately mM concentration and activated only very slightly, if at all, in the range 5–10 mM. This meant that over this range the Mg-ATPase activity

Table 1. *Reversal of effect of DNP on the myofibrillar ATPase*

The myofibrils (11 days old) were assayed for ATPase under the conditions indicated, dialysed, and assayed again with the additions shown. The enzyme incubation medium contained 0.05M tris buffer (pH 7.3), 5 mM-CaCl₂ and 5 mM ATP in all cases. Dialysis was carried out against KCl-borate for 16 hr. at 0° with three changes. Results are expressed as μ g. of P liberated/min./ml. of myofibril suspension at 20°.

Additions to assay medium, before dialysis	ATPase activity after dialysis					
	No added KCl			0.2M-KCl		
	- DNP	+ DNP	% inhibition by DNP	- DNP	+ DNP	% stimulation by DNP
None	37.1	30.1	19	24.2	44.1	82
5 mM DNP (produced 13% inhibition)	41.5	30.8	26	23.7	47.6	101
0.2M-KCl	41.0	30.9	25	22.7	48.5	103
5 mM DNP + M-KCl (produced 220% stimulation)	45.2	36.0	20	26.1	52.4	101

Fig. 4. Effect of DNP on the Ca-ATPase of (a) myofibrils (7 days old) and (b) L-myosin, at varying ionic strengths. Incubations were carried out for 60 min. at 0°. Conditions otherwise the same as those of Fig. 1. Activity in absence of DNP and KCl was taken as 100. ○, No added KCl; ■, 0.1 M-KCl; □, 0.2 M-KCl; △, 0.4 M-KCl; ●, 0.8 M-KCl.

fell by a factor of 50 or 60 on reducing the temperature from 20° to 0°, whereas, in contrast, the Ca-ATPase activity of myofibrils was decreased by a factor of 5 or 6 and the pattern of activation remained unchanged. The results of these experiments are summarized in Fig. 5, as are experiments carried out at pH 7.8 which clearly indicate that the effect on the characteristics of magnesium activation is due to temperature and not to the pH shift obtained on cooling the buffer (see previous section).

Mitochondrial ATPase

Whole mitochondria. It is well known (Kielley & Kielley, 1951) that freshly prepared liver mitochondria hydrolyse ATP very slowly, but that on the addition of 0.1 mM DNP the ATPase activity rises to a high value (the so-called latent ATPase). This effect is obtained with DNP approximately 10–50 times more dilute than that required to produce stimulation of the ATPase of myosin or myofibrils, but there appear to be fundamental similarities in the two systems. To throw light on these the effect of temperature on the DNP stimulation of the mitochondrial ATPase was investigated.

Fig. 5. Activation of the myofibrillar ATPase by magnesium at 0° and 20°. Incubation was carried out in 0.05 M tris buffer, 5 mM ATP. Myofibrils, 3 days old. ○, Incubated at 20° for 5 min. at pH 7.4; ●, incubated at 20° for 5 min. at pH 7.8; □, incubated at 0° for 30 min. at pH 7.4.

Table 2. *Action of DNP on the ATPase activity of fresh liver mitochondria and aqueous extracts of acetone-dried powders*

The incubation medium contained 0.05M tris buffer, 5 mM ATP, and in the experiments with whole mitochondria, 0.1M-KCl to maintain tonicity. All pH values were measured at 20° and results are expressed as $\mu\text{g. of P}$ liberated/min. by 1 ml. of enzyme preparation.

Additions	Whole mitochondria			Acetone-dried powder		
	pH 7.3		pH 7.8	pH 7.4		pH 7.8
	20°	0°		20°	0°	
Nil	1.8	0.2	4.6	4.6	0.6	3.9
0.1 mM DNP	160	2.4	130	5.8	0.5	5.9
5 mM-MgCl ₂	12	1.2	18	18.2	0.9	22.5
0.1 mM DNP + 5 mM-MgCl ₂	146	3.4	142	25.3	0.9	32.4

Fig. 6. Activation of ATPase of an aqueous extract of liver mitochondrial acetone-dried powder. Conditions the same as those of Fig. 5. ○, Incubated at 20° for 5 min. at pH 7.4; ●, incubated at 20° for 5 min. at pH 7.8; ■, incubated at 0° for 30 min. at pH 7.4.

Table 2 shows that at 0° the DNP stimulation of the ATPase of whole rat-liver mitochondria is very much reduced. This effect was obtained in the absence or presence of MgCl₂ in the incubation medium. After pre-incubation with 0.4 mM DNP for 30 min. at 0° the mitochondria were activated on subsequent incubation at 20° in a manner identical with control mitochondria not pre-incubated. Similar effects were obtained with pigeon breast-muscle mitochondria, although it should be noted that in general these mitochondria have appreciable ATPase activity when freshly prepared and in consequence the extent of stimulation by DNP is not so great (Chappell & Perry, 1954).

If the mitochondria were pre-incubated with 0.4 mM DNP under the conditions of the enzyme test, i.e. approximately isotonic, at 0° or 20°, the DNP could be removed by centrifuging and washing with isotonic solutions in the cold without activa-

tion of the ATPase. Subsequent addition of DNP produced reactivation of such DNP-treated mitochondria, although in some cases the activated ATPase level attained was not so high as that obtained with control mitochondria which had not been initially treated with DNP. This suggests that DNP can cause some irreversible inactivation of the enzyme.

Acetone-dried powder. In view of the complexity of the mitochondrial structure compared with that of the myofibril and the possible importance of permeability effects in the study of the ATPase activity of the former, direct comparison of the ATPase activity of these two systems is difficult. A more satisfactory mitochondrial ATPase system for this purpose is the aqueous extract of the acetone-dried powder of mitochondria which has been reported by Lardy & Wellman (1953) to possess ATPase activity stimulated by DNP.

Whereas it was not necessary to add MgCl₂ to whole liver mitochondria to obtain full activity in the presence of DNP, with the acetone-dried powder extract MgCl₂ was essential for full activity (Table 2). As with the myofibril, the mitochondrial ATPase was not activated by magnesium at 0° (Table 2 and Fig. 6), indicating that a high temperature coefficient between 0° and 20° is characteristic of both magnesium-activated ATPases. Also, as might be expected, DNP did not activate the mitochondrial Mg-ATPase at 0° (Table 2). This suggests that the lack of effect of DNP on the ATPase of whole mitochondria at 0° may be in part due to the fact that magnesium does not activate at this temperature. Likewise manganese was found to produce little activation at 0°, whereas at 20° it was at least as effective as magnesium in this respect.

DISCUSSION

The evidence presented indicates that at low ionic strength actin interacts with myosin in a manner which prevents DNP from producing its stimulating effect on the myosin ATPase; in fact, at very low ionic strength DNP is inhibitory to the actomyosin-

ATPase system. The mechanism of DNP stimulation of ATP hydrolysis is obscure, but in view of the effects obtained in the presence of actin it is reasonable to presume that this protein can in some way modify the nature or sequence of events at the enzymically active centres of the myosin. The view that the mechanism of ATP hydrolysis may be modified by actin is also supported by the well-known fact that at low ionic strength magnesium activates the ATPase activity of actomyosin but not of myosin (Banga & Szent-Györgyi, 1943). It is tempting to suppose that there are two slightly different mechanisms for the hydrolysis of ATP by myosin systems, namely one that involves calcium only and another in which either calcium or magnesium can participate. Both mechanisms have many common features, but whereas the former is typical of myosin over a wide range of ionic strength and of actomyosin at ionic strength above 0.15–0.20, the latter occurs in the presence of actin at ionic strengths lower than 0.15–0.20. It is suggested that only the former is stimulated by DNP. Bearing pertinently on this point is the finding that DNP begins to stimulate the ATPase of myofibrils at just that range of ionic strength at which magnesium activation disappears.

It is not clear what changes are induced in the actomyosin system when the ionic strength rises above 0.15–0.20, but certainly the activation properties characteristic of actomyosin disappear and those of myosin remain. It is unlikely that complete dissociation of the actomyosin takes place, but probably there occurs some 'loosening' of the interaction which modifies the state of affairs in the vicinity of the active centres of the myosin so that the influence of the actin moiety is lost.

It is important to decide whether the action of DNP on myosin ATPase presents a model for the study of the action of this phenol on the hydrolysis of ATP by isolated mitochondria, in view of the possible relation of the latter system to oxidative phosphorylation. The extent of stimulation of the ATPase of intact liver mitochondria by DNP is very much greater than that obtained with myosin or myofibrils. It is very questionable, however, if the two systems are strictly comparable, since the extremely low ATPase activity of fresh intact liver mitochondria may be due to the impermeability of the mitochondrial membrane to ATP (see Siekevitz & Potter, 1955). Certainly with isolated pigeon breast-muscle mitochondria, which have high ATPase activity when freshly prepared, and with aqueous extracts of acetone-dried powders of liver mitochondria, the extent of activation is much more comparable to that obtained with the myosin system.

A difference exists in that optimum activation of the myosin ATPase system is obtained with DNP

concentrations 10–50 times as great as those effective on the mitochondrial ATPase. This fact might imply a difference in mechanism of action, but alternatively it may be the result of a difference in affinity of the two systems for DNP. In systems splitting ATP at comparable rates there would be much less mitochondrial ATPase protein than myosin because of the relatively low specific activity of the latter. It is possible also that the relative affinity of the two systems for DNP is determined by the high lipid content of the mitochondrial preparations.

Another point of difference is that the Mg-ATPase of mitochondria is stimulated by DNP, whereas with myosin the Ca-ATPase is the one which is stimulated. Despite this fact the magnesium-activated systems of both mitochondria and myosin are similar in that magnesium activates both systems little or not at all at 0°. Hasselbach (1952) and Gilmour & Calaby (1952) have also commented on the high temperature coefficient of magnesium-activated actomyosin and insect-muscle apyrase respectively. It would be of interest to investigate the effect of temperature on other enzyme systems requiring magnesium and ATP to determine whether this observation is a general one for such systems and reflects perhaps certain features of the complexing action of magnesium for ATP.

SUMMARY

1. In confirmation of Webster's (1953) finding, 2:4-dinitrophenol has been shown to stimulate the calcium-activated adenosine triphosphatase (ATPase) of myosin. The stimulation amounted to 100–150% under optimum conditions, and at 20° occurred when the ionic strength was varied over the range of approx. 0.06–0.46.

2. When actin was present in a medium of low ionic strength, 2:4-dinitrophenol inhibited the calcium-activated ATPase of myosin. 2:4-Dinitrophenol stimulated the calcium-activated ATPase of myofibrils at ionic strength of about 0.16 and higher.

3. At 0° the ionic strength had to be raised to about 0.4 and higher to obtain stimulation of the calcium-activated ATPase activity of L-myosin and myofibrils by 2:4-dinitrophenol.

4. The pattern of calcium activation of the myofibrillar ATPase was similar at 0° and 20°. In contrast, whereas magnesium strongly activated at 20°, at 0° it produced little or no activation of rabbit-myofibrillar or rat-liver mitochondrial ATPases.

5. Some effects of the action of 2:4-dinitrophenol on the rabbit-myofibrillar and the rat-liver mitochondrial ATPases have been compared and discussed.

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Ultraviolet Fluorescence of the Aromatic Amino Acids

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Compounds resembling the aromatic amino acids are known to show appreciable fluorescence in the near ultraviolet [Ley & Englehardt (1910), Kowalski (1911), Marsh (1924)], but the fluorescence of the aromatic amino acids themselves has not so far been unequivocally characterized. Debye & Edwards (1952) have made observations of the phosphorescence of the aromatic amino acids. The position of the phosphorescence bands and the decay times found by these authors indicate the probable existence of fluorescence bands in the near ultraviolet with normal decay times (McClure, 1949).

It is shown in this paper that the three aromatic amino acids exhibit characteristic ultraviolet fluorescence. The fluorescence-excitation spectra, fluorescence spectra and quantum yields of aqueous solutions have been studied. Further papers will deal with aspects of the ultraviolet fluorescence of peptides and proteins.

Characterization of a substance as a fluorescent entity

It is often necessary to determine whether the fluorescence shown by a solution is due to a given substance present in it. To ascribe the observed

fluorescence to a given component of the system the following criteria are proposed:

Fluorescence-excitation spectrum. The quantum yield of the fluorescence of a substance in solution, defined as the ratio of the number of quanta emitted to the number of quanta absorbed, is known to be independent of the exciting wavelength, at least for excitation with light in the air ultraviolet ($\lambda > 2000\text{\AA}$) and visible regions of the spectrum (Wavilov, 1927; Neporent, 1947; Weber & Teale, in preparation). Therefore,

$$F(\lambda) = kqA(\lambda), \quad (1)$$

in which $F(\lambda)$ is the fluorescence intensity set up by excitation with light of wavelength λ , $A(\lambda)$ the number of photons absorbed in the solution, q the quantum yield and k a constant depending on the general geometry of the system and the distribution of the intensity along the exciting beam. A solution of optical density $E(\lambda)$ illuminated with light of this wavelength of intensity $I(\lambda)$ photons absorbs

$$A(\lambda) = I(\lambda) (1 - 10^{-E(\lambda)}). \quad (2)$$

From (1) and (2)

$$F(\lambda) = qkI(\lambda) (1 - 10^{-E(\lambda)}). \quad (3)$$