SPECTROPHOTOMETRIC INVESTIGATION OF THE INTERACTION OF GLUTATHIONE WITH MALEIMIDE AND N-ETHYLMALEIMIDE

by

E. FRIEDMANN

Department of Radiotherapeutics, Cambridge University, Cambridge (England

INTRODUCTION

FRIEDMANN, MARRIAN AND SIMON-REUSS have shown that the unsaturated imides, maleimide and N-ethylmaleimide are inhibitors of mitosis in tissue cultures of chick fibroblasts[1]. The study of sulphydryl adducts of these compounds gave surprising results with glutathione as an –SH donor. It was observed that the dilutions used in tissue culture exerted apparently different antimitotic activities according to the method of their preparation. When prepared after interaction of glutathione with the unsaturated imides had taken place at $M/50$, decreased antimitotic activity, compared with the antimitotic activity of the original imides, was found, whilst the dilutions obtained from $M/1000$ were as active as the original imides[2].

In view of the attention paid in biochemical work to the reactions of –SH compounds an investigation of the mode of the interaction of glutathione with unsaturated imides seemed to be of interest. For this purpose the first approach was a study of the solutions obtained from the interaction of these substances alone and under conditions similar to those present in the biological medium. This was possible as maleimide and N-ethylmaleimide gave characteristic absorption spectra which could be used as standards of referenze for their derivatives. The results obtained are described in the following paper.

METHODS

The solutions resulting from the interaction of the unsaturated imides with glutathione were investigated in Beckman's photoelectric spectrophotometer. It was necessary to use glass distilled water for preparing the dilutions. In order to minimise the occurrence of experimental errors pipettes and measuring flasks used in the higher concentrations were signed and kept apart from those used for the lower concentrations.

The phosphate buffer which has been added in many experiments to be described has been prepared according to COLE[3]. The pH was 7.4 approximately. It will be referred to as phosphate buffer.

The graphs given have been checked repeatedly.

References p. 75.

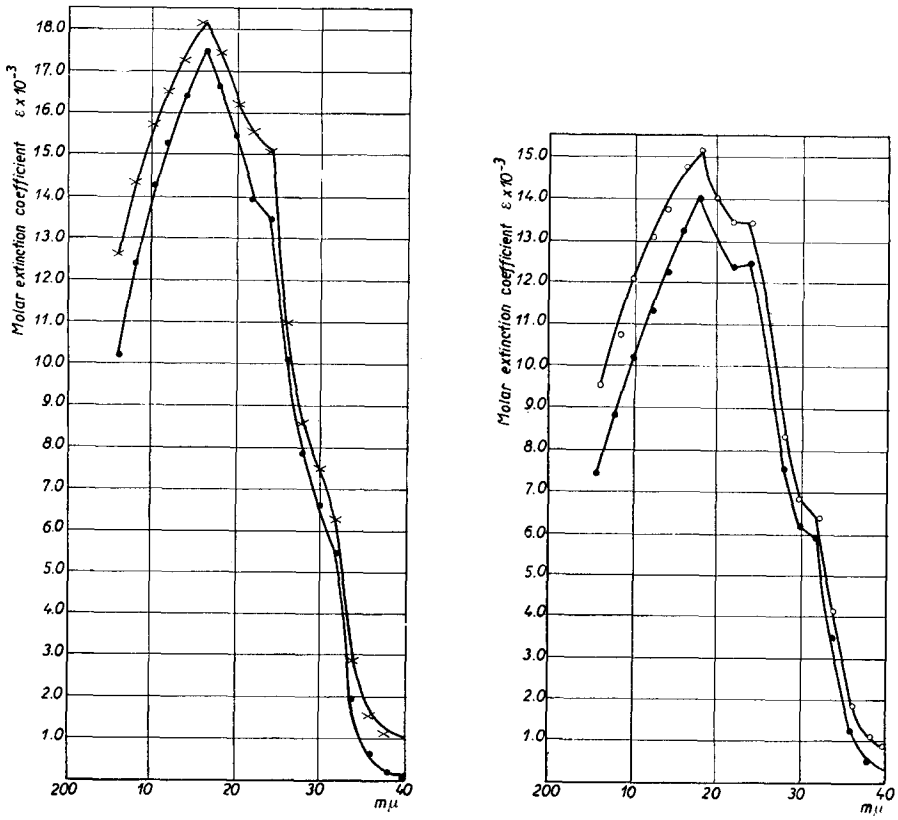


Fig. 1. Absorption spectra of maleimide and of N-ethylmaleimide

Maleimide

$c_m = 4 \cdot 10^{-5}$ ●——● $d = 10$ cm

$c_m = 4 \cdot 10^{-6}$ ×——× $d = 10$ cm

N-Ethylmaleimide

$c_m = 4 \cdot 10^{-5}$ ●——● $d = 10$ cm

$c_m = 4 \cdot 10^{-6}$ ○——○ $d = 10$ cm

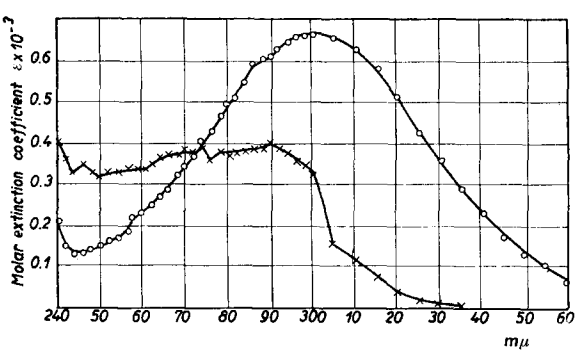
Molar extinction coefficient $\varepsilon \cdot 10^{-3}$ of maleimide ×——×

$$c_m = \frac{0.388}{97} \text{ for } 240\text{–}250 \text{ m}\mu$$

and of N-ethylmaleimide ○——○

$$c_m = \frac{0.05}{125} \text{ for } 234\text{–}250 \text{ m}\mu$$

Solvent H_2O $d = 1$ cm



RESULTS

Absorption spectra of maleimide and N-ethylmaleimide

The absorption curves of maleimide and N-ethylmaleimide are given in Fig. 1 for $c_m = 4 \cdot 10^{-5} M$, $d = 1$ cm. and for $c_m = 4 \cdot 10^{-6} M$, $d = 10$ cm. The solvent was water.

The absorption curve of maleimide is characterized by the steep ascending part of the U.V., reaching its maximum at 216 mμ, the drop from 216–240 mμ, a shoulder at 222–224 mμ and a kink at 232 mμ. N-ethylmaleimide shows a very similar absorption curve with a peak at 218 mμ and two shoulders in the descending part of the curve, the one at 222–224 mμ the other at 230–232 mμ. ε_{max} is higher for maleimide than for N-ethylmaleimide, $\varepsilon \cdot 10^{-3}$ being, in the experiment described, 18.2 for maleimide and 15.2 for N-ethylmaleimide ($c_m = 4 \cdot 10^{-6} M$). Neither substance follows Beer's Law, as shown by the different molar extinction coefficients at $4 \cdot 10^{5} M$ and $4 \cdot 10^{6} M$.

For reasons of completeness the molar extinction coefficient of maleimide and N-ethylmaleimide between 240 and 350 mμ are added, although the evidence presented in the following paragraphs is based alone on the molar extinction coefficient of these substances, observed between 216 and 240 mμ.

Interaction of neutralised glutathione at $1.5 \cdot 10^{-4} M$ with $4 \cdot 10^{-6} M$ unsaturated imides

In tissue culture experiments the unsaturated imides were tested by applying to the growing tissue a solution of the imide at a certain concentration, mixed with an equal volume of plasma, containing embryo extract to give a final concentration of 15% embryo extract. Embryo extract is rich in glutathione, its content being as high as 30 mg%[4]. This corresponds to a molar concentration of $\sim 1 \cdot 10^{-3} M$. The molar concentration of glutathione in the plasma/embryo extract, corresponding to a final concentration of 15% embryo extract, is therefore $1.5 \cdot 10^{-4} M$. In order to see whether interaction of glutathione with a solution of unsaturated imide at a concentration, shown to be active as an inhibitor of mitosis in tissue culture, has taken place, a neutralised solution of glutathione at $1.5 \cdot 10^{-4} M$ was brought into contact with a solution of an unsaturated imide at $4 \cdot 10^{-6} M$. As glutathione reacts nearly instantaneously and completely with unsaturated imides, the reaction fluids could be investigated shortly after mixing.

In Fig. 2 the results are collected of the interaction of neutralised glutathione at $1.5 \cdot 10^{-4} M$ with maleimide and N-ethylmaleimide, both at $4 \cdot 10^{-6} M$. The solutions were buffered with phosphate buffer prepared according to COLE, to give a 10^{-3} M phosphate solution. The concentrations are final concentrations.

In graphs 1 and 2 of Fig. 2 the absorption resulting from the interaction of glutathione with maleimide and N-ethylmaleimide are compared with the absorption curves of these unsaturated imides; in graph 3 they are compared with the absorption curves of glutathione. Graph 3 demonstrates that in this experiment the absorption curves of maleimide plus glutathione and of N-ethylmaleimide plus glutathione are nearly identical and that both present only small differences from the absorption curve of glutathione. As the absorption curve of glutathione dominates at the concentrations used, the picture of the absorption curves, it is reasonable to assume that the strong absorption, shown in graphs 1 and 2, is due to glutathione. This result is easily understood, if one considers that at a concentration of $4 \cdot 10^{-6} M$ of the imides, and at $1.5 \cdot 10^{-4} M$ of glutathione, only $1.5 \cdot 10^{-4} - 4 \cdot 10^{-6}$ mole glutathione, or 2.7% of the total glutathione present, have been used up for the interaction of the imides with glutathione.

References p. 75.

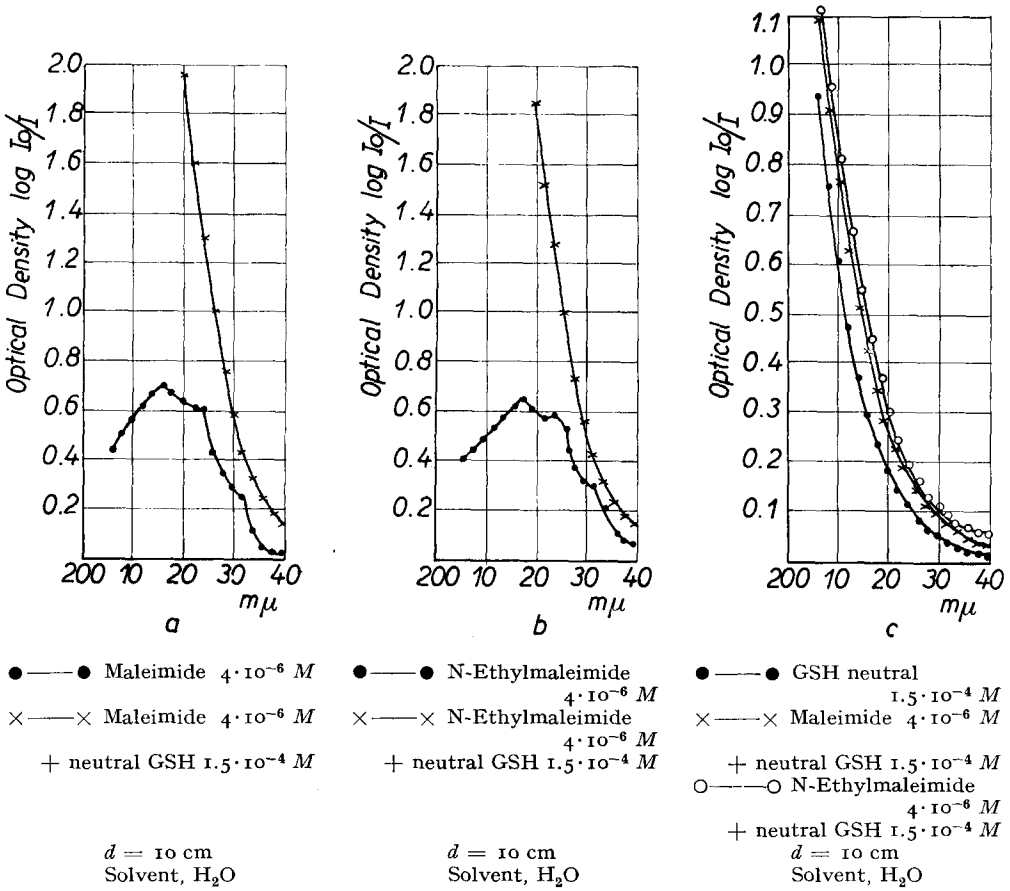


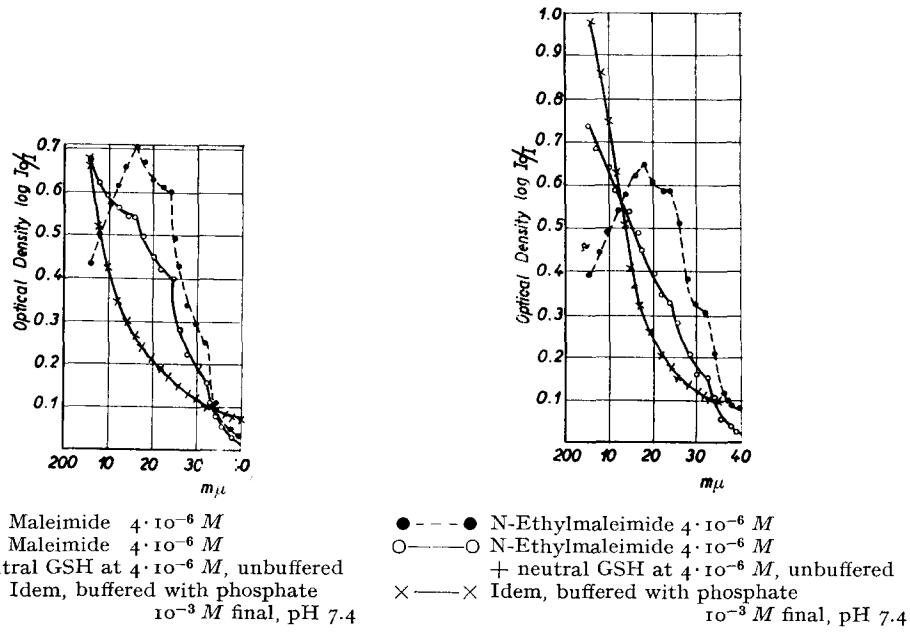
Fig. 2. Interaction of neutralised glutathione (GSH) at $1.5 \cdot 10^{-4}$ *M* with maleimide and N-Ethylmaleimide both at $4 \cdot 10^{-6}$ *M* in phosphate buffer (10^{-3} *M* final, pH 7.4)

Interaction of neutralised glutathione at $4 \cdot 10^{-6}$M with maleimide and N-ethylmaleimide at $4 \cdot 10^{-5}$ and $4 \cdot 10^{-4}$M concentrations in buffered solutions

The amount of unchanged glutathione present in the interaction of $1.5 \cdot 10^{-4}$*M* glutathione with $4 \cdot 10^{-6}$*M* unsaturated imides made it impossible to ascertain whether a reaction has taken place. Assuming that the reaction under discussion has followed a normal course, only $4 \cdot 10^{-6}$ moles glutathione could have reacted with $4 \cdot 10^{-6}$ moles unsaturated imides. It seemed therefore advisable to discard the amount of glutathione which does not take part in the reaction at $4 \cdot 10^{-6}$*M*. Another point was examined at the same time. The unsaturated imides were added in tissue cultures to embryo extract and thus to glutathione in unbuffered medium at neutral reaction, whilst the addition in the chemical model experiments took place at pH 7.4 in a fluid strongly buffered with phosphate. Buffered and unbuffered solutions of the reactants were therefore compared. The concentration of the unsaturated imides which is important for the problem under examination is $4 \cdot 10^{-6}$*M*. The reaction taking place at a concentration of $4 \cdot 10^{-6}$*M* has

References p. 75.

been compared with the reactions observed under the same conditions at $4 \cdot 10^{-5}$ and at $4 \cdot 10^{-4}$ M. Fig. 3 shows the results of the interaction of neutralised glutathione of $4 \cdot 10^{-6} M$ with maleimide and N-ethylmaleimide, both at $4 \cdot 10^{-6} M$, in buffered and unbuffered solutions. In Fig. 4 the same reaction is represented, but the concentration of the unsaturated imides are raised to $4 \cdot 10^{-5} M$ and $4 \cdot 10^{-4} M$ respectively.



●- - -● Maleimide $4 \cdot 10^{-6}$ M
O——O Maleimide $4 \cdot 10^{-6}$ M
\+ neutral GSH at $4 \cdot 10^{-6}$ M, unbuffered
×——× Idem, buffered with phosphate 10^{-3} M final, pH 7.4

●- - -● N-Ethylmaleimide $4 \cdot 10^{-6}$ M
O——O N-Ethylmaleimide $4 \cdot 10^{-6}$ M
\+ neutral GSH at $4 \cdot 10^{-6}$ M, unbuffered
×——× Idem, buffered with phosphate 10^{-3} M final, pH 7.4

Fig. 3. Interaction of neutralised glutathione at $4 \cdot 10^{-6}$ M with maleimide and N-ethylmaleimide, both at $4 \cdot 10^{-6}$ M in buffered and unbuffered solutions

The absorption curves recorded in Fig. 3 show clearly that at $4 \cdot 10^{-6} M$ concentrations of glutathione and of $4 \cdot 10^{-6}$, $4 \cdot 10^{-5}$ and $4 \cdot 10^{-4}$ M respectively of maleimide and N-ethylmaleimide reaction has taken place. At $4 \cdot 10^{-6} M$ concentration of the imides and of glutathione the influence of the phosphate buffer is apparent, as seen from Fig. 3. The reaction product from unbuffered glutathione, neutralised against Litmus, is different from the substance present in phosphate buffer, both compared with the original absorption curves of maleimide and N-ethylmaleimide. The absorption curves derived in unbuffered neutral solutions from these substances are flatter and have both lost the ascending part leading in the U.V. to the maximum at 216 and 218 mμ respectively, but they have kept the characteristic shoulders at 224 and 232 mμ. The maleimide curve has regularly a kink at 216 mμ, the N-ethylmaleimide curve occasionally a shoulder at 218 mμ, the wavelengths of ε_{max} in the original imides. In the absorption curves of the substances formed in phosphate buffer the curvature is reversed. The curves have lost completely, or nearly completely, the characteristic features of the original imides. The absorption of the glutathione adduct of N-ethylmaleimide is at 206 mμ considerably stronger than the corresponding absorption of the maleimide adduct.

In control experiments the absorption spectra of maleimide and N-ethylmaleimide

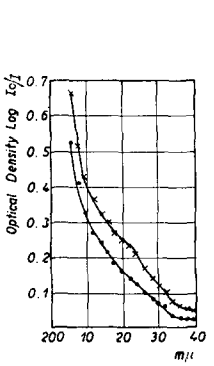
References p. 75.

both at $4 \cdot 10^{-6} M$ in phosphate buffer, giving a final buffer concentration of $10^{-3} M$ were examined. The unchanged absorption spectra of maleimide and N-ethylmaleimide were observed.

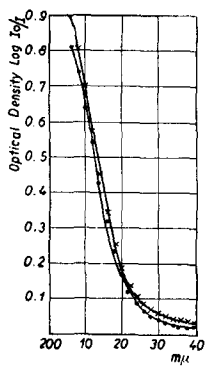
In Fig. 3 the absorption curves of the three substances, the original imides, the adducts with glutathione in phosphate buffer and the substances obtained from unbuffered neutralised glutathione, are compared. In the N-ethylmaleimide experiment the three curves are intersecting at 213 mμ. In four other experiments intersection was observed at 212, 210, 212, 212 mμ.

Fig. 4 gives the results of the interaction of glutathione with maleimide and N-ethylmaleimide at $4 \cdot 10^{-5} M$ and $4 \cdot 10^{-4} M$ in buffered and unbuffered solutions. It will be seen that the interesting substance which disclosed its presence in the unbuffered solution at $4 \cdot 10^{-6} M$ concentration of the reactants is no more in evidence. The absorption curves of the buffered and unbuffered solutions resulting from the N-ethylmale-

A. Reaction carried out at $4 \cdot 10^{-5}$ *M*. Resulting fluid diluted to $4 \cdot 10^{-6}$ *M*

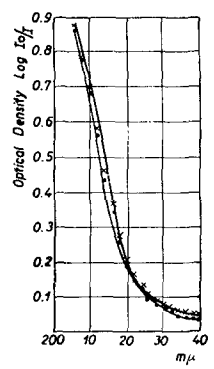


Maleimide
d = 10 cm Solvent, H_2O

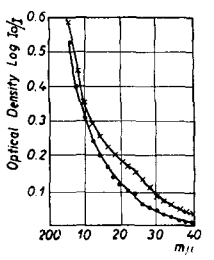


N-Ethylmaleimide
d = 10 cm Solvent, H_2O

B. Reaction carried out at $4 \cdot 10^{-4}$ *M*. Resulting fluid diluted to $4 \cdot 10^{-6}$ *M*



Maleimide
d = 10 cm Solvent, H_2O



N-Ethylmaleimide
d = 10 cm Solvent, H_2O

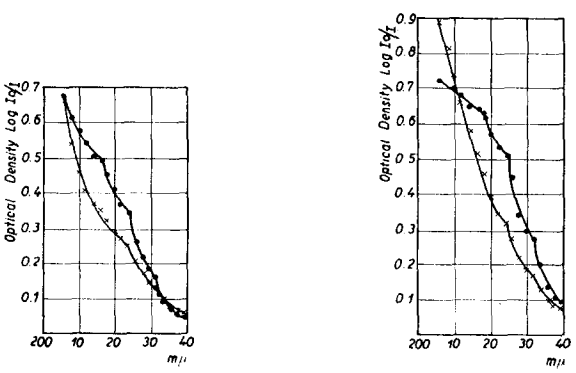
Fig. 4. Interaction of neutralised glutathione with maleimide and N-ethylmaleimide at $4 \cdot 10^{-5} M$ and $4 \cdot 10^{-4} M$ in buffered and unbuffered solutions. ●——● unbuffered solutions; ×——× buffered solutions (phosphate pH 7.4, $10^{-3} M$ final)

References p. 75.

imide reaction are so much alike, that no influence of the addition of buffer to the solution can be recognised in the course of the reaction. But the corresponding maleimide curves show slight differences between the buffered and unbuffered solutions which may have some significance.

Relation of the substances resulting from the reaction of glutathione, maleimide and N-ethylmaleimide at $4 \cdot 10^{-6} M$ concentrations in unbuffered solutions to the substances obtained in phosphate buffer

The investigation of the reaction of glutathione with maleimide and N-ethylmaleimide at $4 \cdot 10^{-6} M$ has shown that in unbuffered solutions and in buffered solutions different substances are formed. The resemblance of the absorption curves of the substances obtained in unbuffered solutions with the original imides could be interpreted by assuming that these compounds are first reaction products, which on addition of phosphate buffer give the final adducts, deprived partially or completely of their resemblance with the starting material. This assumption was tested by allowing the unsaturated imides to react woth glutathione in unbuffered solutions at $4 \cdot 10^{-6} M$, and after reaction had taken place by diluting the reaction fluid to $3.6 \cdot 10^{-6} M$. In one case water has been used for the dilution, in the other case phosphate buffer was added to give $10^{-3} M$ final concentration of the phosphate buffer. The solution obtained through dilution with water showed the characteristic absorption curve, observed regularly in the unbuffered, neutral reaction mixture, whilst the addition of the phosphate buffer gave rise at once to the very different absorption curve of the adduct. The experiment recorded in Fig. 5 demonstrates clearly that the substances obtained from the interaction of neutralised, unbuffered glutathione with maleimide and N-ethylmaleimide at $4 \cdot 10^{-6} M$ are precursors of the adducts found in phosphate buffer.



Maleimide N-Ethylmaleimide

Fig. 5. Reaction of unbuffered neutralised glutathione with maleimide and N-ethylmaleimide $4 \cdot 10^{-6} M$ investigated after dilution to $3.6 \cdot 10^{-6} M$

●——● dilution with water

×——× dilution with phosphate buffer pH 7.4 to give a final buffer concentration of $10^{-3} M$

Spectrophotometric investigation of dilutions prepared after interaction of glutathione with maleimide and N-ethylmaleimide at M/50 and M/1000 of the reactants

In the experiments recorded so far the interaction of glutathione with maleimide and N-ethylmaleimide has been studied at $4 \cdot 10^{-4}$, $4 \cdot 10^{-5}$ and $4 \cdot 10^{-6} M$ of the reactants.

References p. 75.

In the biological experiments the reaction was carried out at $M/50$ and $M/1000$, and the so obtained reaction fluids were diluted to $4 \cdot 10^{-6} M$ or to another dilution of the same order. These two different ways of preparing the fluids to be tested in tissue cultures gave different biological results. As it was possible that in a concentrated solution, as in $M/50$, another equilibrium was produced, as in a dilute solution such as in $M/1000$, a spectrophotometric investigation of the dilutions, prepared after reaction of glutathione with maleimide and N-ethylmaleimide at $M/50$ and $M/1000$ of the reactants, was carried out.

In Table I the optical densities observed in four experiments are recorded, after interaction of maleimide and N-ethylmaleimide at $M/50$ and $M/1000$ respectively, and dilution of the reaction fluids to $4 \cdot 10^{-6} M$.

TABLE I

OPTICAL DENSITIES, OBSERVED AT $4 \cdot 10^{-6} M$, $d = 10$ cm

Solvent: H_2O. t = 22°

mμ	*Interaction of Maleimide with glutathione both at:*		*Interaction of N-ethylmaleimide with glutathione both at:*	
	$M/50$	$M/1000$	$M/50$	$M/1000$
206	0.524	0.491	0.818	0.802
208	0.401	0.379	0.747	0.711
210	0.306	0.293	0.650	0.419
212	0.241	0.235	0.540	0.516
214	0.195	0.188	0.426	0.410
216	0.165	0.157	0.317	0.310
218	0.140	0.135	0.230	0.227
220	0.118	0.114	0.165	0.165
222	0.104	0.098	0.119	0.119
224	0.088	0.085	0.086	0.090
226	0.075	0.071	0.067	0.070
228	0.065	0.068	0.055	0.055
230	0.057	0.054	0.045	0.046
232	0.051	0.048	0.037	0.038
234	0.045	0.043	0.034	0.034
236	0.040	0.039	0.030	0.030
238	0.038	0.036	0.029	0.029
240	0.036	0.035	0.026	0.025

It will be seen from Table I that the optical densities observed at $4 \cdot 10^{-6} M$ show between 218 and 240 mμ good agreement independently, whether the reactions at $M/50$ or the reactions at $M/1000$ have been used as starting points for the preparation of the final dilutions of $4 \cdot 10^{-6} M$. In contrast, the agreement between 206 and 216 mμ is less satisfactory and in spite of numerous further experiments no clear picture could be worked out, proving or disproving the occurrence of different equilibrium mixtures, resulting from the initial reactions at $M/50$ and $M/1000$ concentration.

DISCUSSION

Two problems have been approached in the preceding experiments. It has been

References p. 75.

attempted to explain how in tissue cultures the antimitotic activity of the unsaturated imides, maleimide and N-ethylmaleimide, remained the same in the absence and in the presence of added glutathione, and further, it was attempted to find an answer to the surprising observation that the mitotic inhibition of unsaturated imides in presence of glutathione was dependant on the concentrations which served as starting points for preparing the dilutions to be tested.

The answer for the first problem was found by showing that the content of glutathione in embryo extract was so high that from the total amount of glutathione present in the plasma/embryo mixture only 2.7% was necessary to form the adduct with the unsaturated imide at $4 \cdot 10^{-6} M$ concentration. In addition, it was demonstrated spectroscopically that at $4 \cdot 10^{-6} M$ reaction takes place between glutathione and the unsaturated imides, thus proving that the antimitotic activity observed after adding the unsaturated imides to the embryo extract is in reality the activity of the adduct. It is therefore easily understood that the mitotic inhibition of the unsaturated imides, maleimide and N-ethylmaleimide, remains in tissue cultures the same in the absence and in the presence of added glutathione.

The observation that in neutral unbuffered solutions of the reactants at $4 \cdot 10^{-6} M$ a substance is formed which is different from the adduct obtained in phosphate buffer gave for a time some hope of explaining the quantitative differences of the inhibitions caused by unsaturated imides depending upon the concentrations from which the examined solution was prepared. This hope vanished quickly as it was found that the mere addition of phosphate buffer to the first substance changed it into the second, and that at higher concentrations, $4 \cdot 10^{-5} M$ and $4 \cdot 10^{-4} M$, the first substance was not to be found. For the concentration $4 \cdot 10^{-6} M$ of the reactants it was possible to separate the glutathione/imide reaction into a sequence of two reactions, both proceeding with great velocity to completion. With maleimide and N-ethylmaleimide it is therefore hopeless to try to advance the question by studying the reversibility of an equilibrium, giving 98–100% of the final adduct in a sequence of two reactions within two minutes. But the glutathione citraconimide reaction comes to a standstill after 75% of the glutathione has been taken up, and here equilibrium investigations may advance the problem. They will be carried out when citraconimide has been made more easily available.

It has been mentioned that the absorption curves of the three substances, the original imide, the intermediate and the final product, show intersection in the region of 212 mμ. As two of the substances taking part in this intersection do not obey Beer's law the intersection point cannot be classified, as isosbestos point, as this name is by definition reserved for the intersection point of substances following Beer's law[5,6]. Nevertheless, the occurrence of a common intersection point contributes to the concept of a close relationship between the three substances taking part in the reaction.

It would be premature to advance any suggestion concerning the chemical nature of the intermediate, although the possibility that it is a molecular compound is kept well in mind.

The interesting observation that the physiological concentration at which the antimitotic activity of the unsaturated imides has been ascertained coincides with the concentration at which the intermediate of the imide-glutathione reaction is stable may be noted, but no conclusions can be drawn from this observation as the great buffering capacity of plasma and embryo extract may shorten the lifetime of this intermediate in the same way as does the addition of phosphate buffer in the chemical experiment.

References p. 75.

The picture developed in the study of the imide/glutathione reaction at $4 \cdot 10^{-6} M$, although far from being complete, is beginning to represent, as it seems coherently, the processes involved at this concentration. This simplification is seriously disturbed by the quantitatively different results obtained by using in tissue culture experiments dilutions prepared after the reaction has taken place at a higher concentration. Spectrophotometric investigations to decide whether at a higher concentration of the reactants the same or other products are found than at lower concentrations failed to produce a clear decision. The absorption curves obtained from solutions derived from higher and lower concentrations of the reactants have not given identical values between 206 and 216 mμ but have shown satisfactory agreement between 218 and 240 mμ. For the time being it is not possible to assess the significance of this discrepancy since the absolute values of the absorption curves obtained in the U.V. with the Beckman spectrophotometer show considerable fluctuations. Similar observations have been made repeatedly by J. S. MITCHELL[7].

The investigation of the glutathione/imide reaction of $4 \cdot 10^{-6} M$ could only be approached by applying spectrophotometric methods to the reaction fluids. In contrast the reactions at $M/50$ and $M/1000$ are open to direct chemical analysis. The isolation of the reaction product, obtained at these concentrations with cysteine as –SH donor, has been carried out and will be continued with glutathione. It is hoped that the direct examination of these adducts will provide the missing information.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation for the skilled technical assistance of Mr JOHN HARRIS. He is greatly indebted to Messrs May and Baker, Ltd, Dagenham, Essex, for financial support.

SUMMARY

The spectrophotometric investigation of the interaction of glutathione with maleimide and N-ethylmaleimide gave the following results:

1. Glutathione interacts with maleimide and N-ethylmaleimide even at $4 \cdot 10^{-6} M$ concentration of the reactants.

2. In the solutions resulting from the interaction of glutathione with maleimide and N-ethylmaleimide at $4 \cdot 10^{-6} M$, different substances were found when the reaction was carried out in an unbuffered, neutralised medium and when it took place in phosphate buffer.

3. The substances obtained in neutralised, unbuffered solutions have not been found at higher concentrations ($4 \cdot 10^{-5}$ and $4 \cdot 10^{-4} M$).

4. The substances found in unbuffered, neutral solutions at $4 \cdot 10^{-6} M$ are the precursors of the substances obtained in phosphate buffer.

5. The significance of these results for the interpretation of some of the observations, encountered with the unsaturated imides in tissue cultures of chick fibroblasts, is discussed.

6. The different antimitotic activities of the unsaturated imide/glutathione mixture, dependent upon the concentration at which the compounds were brought to reaction before dilution, have found no satisfactory explanation, since the spectrophotometric investigation of the equilibrium mixtures, derived from the interaction at $M/50$ and $M/1000$, gave no conclusive results.

RÉSUMÉ

L'examen spectrophotométrique de la réaction entre le glutathion et les imides non saturées, la maléimide et la N-éthylmaléimide a donné les résultats suivants:

1. Le glutathion entre en réaction avec la maléimide et la N-éthylmaléimide même à la concentration moléculaire de $4 \cdot 10^{-6} M$.

References p. 75.

2. Les produits resultants de la réaction entre le glutathion et les imides, la maléimide et la D-éthylmaléimide, à $4 \cdot 10^{-6}$ *M*, sont differents suivant qu'ils ressortent d'une solution tamponnée avec du phosphate à pH 7.4 ou d'une solution neutre, non tamponnée.

3. Les substances, presentes dans les solutions neutres, non tamponnées, n'ont pas été trouvées, si la réaction a été exécutée à des concentrations moléculaires plus élevées ($4 \cdot 10^{-5}$ *M* et $4 \cdot 10^{-4}$ *M*).

4. Les corps, obtenus dans des conditions de réaction neutres et non tamponnées, sont des intermédiaires des produits formés dans les solutions tamponnées.

5. L'application de ces résultats à l'action antimitotique des imides non saturées est discutée.

6. Les différences quantitatives, observées dans l'action antimitotique des mélanges glutathion/imides, et la dépendance de ces différences de la concentration à laquelle le glutathion a réagi avec les imides, n'ont pas trouvé une explication satisfaisante dans les recherches spectrophotométriques presentées.

ZUSAMMENFASSUNG

Die spektrophotometrische Untersuchung der Reaktion zwischen Glutathion und den ungesättigten Imiden, Maleinimid und N-Äthylmaleinimid, ergab die folgenden Resultate:

1. Glutathion reagiert selbst bei einer molaren Konzentration von $4 \cdot 10^{-6}$ *M* mit Maleinimid und N-Äthylmaleinimid.

2. Die Reaktionsprodukte, die erhalten werden, wenn Glutathion mit Maleinimid und N-Äthylmaleinimid bei $4 \cdot 10^{-6}$ *M* in ungepufferter neutraler lösung, und wenn es in Phosphatpuffer vom pH 7.4 zur Einwirkung gebracht wird, sind verschieden.

3. Die in ungepufferter, neutraler Lösung bereiteten Reaktionsprodukte konnten bei höheren Konzentrationen ($4 \cdot 10^{-5}$ *M* und $4 \cdot 10^{-4}$ *M*) nicht aufgefunden werden.

4. Die bei neutraler Reaktion in ungepufferten Lösungen nachgewiesenen Substanzen sind Zwischenprodukte der in gepufferten Lösungen erhaltenen Glutathionaddukte.

5. Die Bedeutung dieser Befunde für die Interpretation der in Gewebekulturen bei der Prüfung der ungesättigten Imide erhaltenen Resultate wurde erörtert.

6. Die quantitativen Differenzen in der antimitotischen Wirkung, die mit Glutathion/Imid Mischungen erzielt worden sind, und ihre Abhängigkeit von der Konzentration, bei der die Substanzen zur Reaktion gebracht worden sind, haben in den mitgeteilten spektrophotometrischen Untersuchungen keine befriedigende Erklärung gefunden.

REFERENCES

[1] E. FRIEDMANN, D. H. MARRIAN, AND I. SIMON-REUSS, *Brit. J. Pharmacol.*, 4 (1949) 105.
[2] E. FRIEDMANN, D. H. MARRIAN, AND I. SIMON-REUSS, *Biochim. Biophys. Acta*, 9 (1952) 61.
[3] S. W. COLE, *Practical Physiological Chemistry*, 9th edition, p. 24.
[4] L. RAPKINE, *Compt. rend.*, 191 (1930) 871.
[5] F. WEIGERT, *Ber.*, 49 (1916) 1525.
[6] W. WEST, in A. WEISSBERGER, *Physical Methods of Organic Chemistry*, Vol. 2 (1946) p. 776.
[7] J. S. MITCHELL. Private communication.

Received September 11th, 1951