Phasenverschiebung kann selbstverständlich erst nach dem Abklingen von Transienten, im sogenannten «steady state», gemessen werden.

Der durch $\Delta\tau$ bedingte Fehler bei den genannten Methoden zur Bestimmung von $\Delta\varphi$ kann in Fall 1 und 2 jeweils maximal den Wert von $\Delta\tau$ selbst erreichen. In Fall 3 überschreitet der Fehler $\Delta\tau/2$ nicht. Die effektive Grösse des Fehlers errechnet sich in allen drei Fällen nach der allgemeinen Formel $-\Delta\tau \cdot \varphi_s$. Hierbei ist $\Delta\tau$ die Frequenzänderung in Minuten und φ_s der Phasenwinkel des Stufenreizes, ausgedrückt als Dezimale von 360° ($x°/360°$). Der Ausdruck ist deshalb negativ, weil ein positives $\Delta\varphi$ einem negativen $\Delta\tau$ entspricht und umgekehrt. In Fall 1 liegt bei gegebenem $\Delta\tau$ das Maximum des Fehlers ($= -\Delta\tau$) bei 0°, also bei Reiz in Punkt A; der Fehler ist Null, wenn φ_s 360° nach Punkt A liegt. Im 2. Fall liegt umgekehrt das Maximum des Fehlers bei 360° und das Minimum bei 0°. Fall 3 weist seinen maximalen Fehler ($-\Delta\tau/2$) mit umgekehrtem Vorzeichen bei 0° und 360° auf; der Fehler ist Null bei $\varphi_s = 180°$. Für die drei Beispiele bisher üblicher $\Delta\varphi$-Bestimmungen gelten entsprechend folgende Formeln zur Eliminierung des $\Delta\tau$-bedingten Fehlers:

1) $\Delta\varphi_c = \Delta\varphi_m + \Delta\tau\,(1 - \varphi_s)$

2) $\Delta\varphi_c = \Delta\varphi_m - \Delta\tau\varphi_s$

3) $\Delta\varphi_c = \Delta\varphi_m + \Delta\tau\,(0{,}5 - \varphi_s)$.

$\Delta\varphi_c$ = das durch Eliminierung des Fehlers korrigierte $\Delta\varphi$

$\Delta\varphi_m$ = das nach der jeweiligen Methode gemessene $\Delta\varphi$.

Beispiel (vgl. Figur 1):

$\left.\begin{array}{l}\tau_1 = 25\text{ h}\\ \tau_2 = 23\text{ h}\end{array}\right\}\ \Delta\tau = -120'$

$\varphi_s = 5$ h ($90° = 90°/360° = 0{,}25$) nach A

$\Delta\varphi_{m1} = -\ 90'$
$\Delta\varphi_{m2} = -\ 210'$
$\Delta\varphi_{m3} = -\ 150'$

$\left.\begin{array}{l}1)\ \Delta\varphi_c = -\ 90' - 120'\,(1 - 0{,}25)\\ \qquad = -\ 90' - \ 90' = -\ 180'\end{array}\right\}$ Fehler $= -\ 90'$

$\left.\begin{array}{l}2)\ \Delta\varphi_c = -\ 210' + 120' \cdot 0{,}25\\ \qquad = -\ 210' + \ 30' = -\ 180'\end{array}\right\}$ Fehler $= +\ 30'$

$\left.\begin{array}{l}3)\ \Delta\varphi_c = -\ 150' - 120'\,(0{,}5 - 0{,}25)\\ \qquad = -\ 150' - \ 30' = -\ 180'\end{array}\right\}$ Fehler $= -\ 30'$

Eine wesentliche Vereinfachung der $\Delta\varphi$-Korrektur lässt sich mit Hilfe graphischer Darstellungen erreichen. Grösse und Vorzeichen des Fehlers können unmittelbar aus dem Diagramm entnommen werden; dieser Wert ist – entsprechend dem doppelt negativen Vorzeichen – zu dem gemessenen Wert $\Delta\varphi_m$ zu addieren. Näheres ist den entsprechenden Legenden zu entnehmen.

Summary. Phase shifts ($\Delta\varphi$) are commonly measured without considering the cue's phase position (φ_s). The normally adopted methods for measuring phase shifts give errorless results only when there is no change in period length ($\Delta\tau$). Very often, however, both phase shifts and changes in period length occur together. The magnitude of the error is then a function of the magnitude of $\Delta\tau$ and the distance between the points of measurement and the cue's phase position. (The basic hypothesis is that $\Delta\tau$ is effected immediately after the cue.) Methods for calculating and eliminating the error are given.

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STUDIORUM PROGRESSUS

Radiation Inactivation of Purified Lysine Vasopressin

A number of protein and polypeptide hormones have been studied in our laboratory by means of the technique of radiation inactivation of biological and immunological activities. These have included human chorionic gonadotropin[1], human pituitary gonadotropins[2], bovine thyrotropin[3], adrenocorticotropin[4], and melanocyte stimulating hormone[4]. In order to further understand the effects of ionizing radiation on such hormones, we have undertaken studies on a polypeptide hormone which is available in completely pure form and whose amino acid sequence and structure are well known – lysine vasopressin. This report presents the data obtained during the course of these studies.

Methods. Detailed discussions of preparation of samples for radiation, radiation and methods of data analysis have been previously published[1,3,4]. Highly purified lysine vasopressin (LVP) was prepared as previously described[5,6]. In one series of experiments, 1 mg of LVP was placed on each planchet or in each bottle. In a second series of experiments, 0.5 mg was placed in each container. Samples were radiated in either an argon atmo-

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sphere at reduced pressure or in air as indicated. This methodology and the radiation dosage (rads) monitored in each case have been described previously[4]. In experimental series A, 8 containers were radiated in argon at each of the following radiation doses: zero, $3.0 \cdot 10^8$, $6.0 \cdot 10^8$, $9.0 \cdot 10^8$, and $12.0 \cdot 10^8$ rads. In a second experimental series (B), 4 samples were radiated in air at each of the following doses: zero, $1.0 \cdot 10^8$, $2.0 \cdot 10^8$, $4.0 \cdot 10^8$, $6.0 \cdot 10^8$, $8.0 \cdot 10^8$, and $12.0 \cdot 10^8$ rads. In experimental series C, 4 or 5 samples were radiated at zero, $3.0 \cdot 10^8$, $6.0 \cdot 10^8$, $9.0 \cdot 10^8$, and $12.0 \cdot 10^8$ rads.

Bioassays. Vasopressor activity was measured in the rat as described previously[7]. All planchets were individually assayed. Each sample was diluted appropriately to place at least 2 doses in the dose response portion of the assay. All doses were assayed in duplicate, and standard dose response curves were performed in all assays.

Amino acid analysis. Identical aliquots of LVP radiated at 2 dosages were hydrolyzed with 6*N* hydrochloric acid at 105 °C for 20 h. Amino acids were analyzed in a Beckman Model 120 amino acid analyzer and the results were compared to non-radiated hormone handled in an identical fashion except that these samples were not passed through the electron beam. The data are expressed as % of amino acid composition of non-radiated hormone which has been reported previously[8].

Results. Radiation inactivation of biological activity. When LVP was radiated in air or in an argon atmosphere, loss of biological activity was observed (Figure). The dose required to reduce activity to 37% (D_{37}) when radiation was performed in air was $2.24 \cdot 10^8$ rads (95% limits $1.91–2.70 \cdot 10^8$). The D_{37} when radiation was performed in the argon atmosphere was $3.43 \cdot 10^8$ rads (2.76 to $4.56 \cdot 10^8$). Assuming each *ionization* occurs within the confines of the ADH molecules[3], the molecular weight may be estimated from the formula:

$$M = \frac{0.24 \cdot 10^{12}}{D_{37}}$$

where M is molecular weight and D_{37} is the radiation dose in rads required to reduce activity to 37%. The M thus calculated was $1.07 \cdot 10^3$ ($9.0 \cdot 10^2$–$1.3 \cdot 10^3$) for the data obtained in air. When radiation was performed in argon, the M was $7.06 \cdot 10^2$ (5.3–$9.0 \cdot 10^2$).

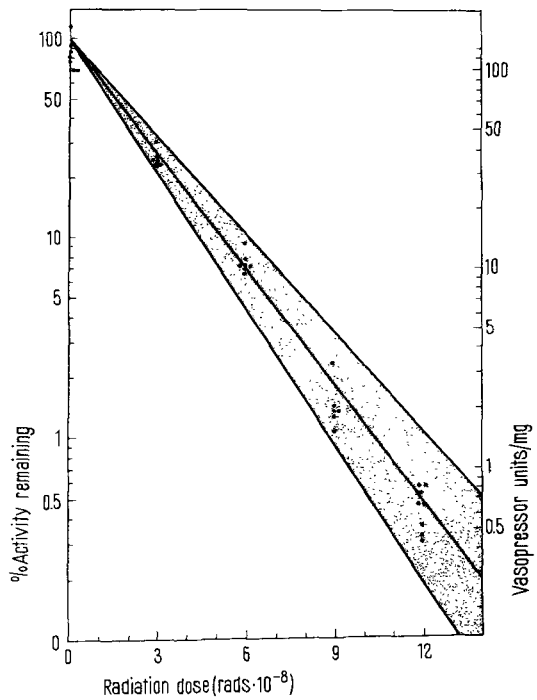
If, as is assumed for larger molecules, each *ionization cluster* occurs within the confines of the molecules, the formula used would be:

$$M = \frac{0.72 \cdot 10^{12}}{D_{37}}$$

and the M calculated from the data in air was $3.0 \cdot 10^3$ (2.7–$3.8 \cdot 10^3$). From the data obtained in argon, the M calculated was $2.1 \cdot 10^3$ (1.6–$2.6 \cdot 10^3$).

Effect of ionization on amino acid content of LVP. Amino acid analyses were performed on identical aliquots of LVP subjected to a 'high' and 'low' dose of radiation. These results were compared to identical samples of non-radiated LVP and are summarized in the Table. At both $2.50 \cdot 10^8$ rads and $10.0 \cdot 10^8$ rads, destruction of amino acids occurred. Cystine was the amino acid destroyed in greatest quantities – over $^2/_3$ of the cystine was destroyed at $10.0 \cdot 10^8$ rads. The data from amino acid analyses are summarized in the Table.

Discussion. The theoretical considerations concerning the technique of radiation inactivation have been discussed in earlier publications[1–4]. Estimation of molecular weight by this technique is independent of density when radiation dose is expressed in rads. Furthermore, purification is not required, since only loss of biological activity need be measured. In the studies described herein, purified LVP was utilized, however, to allow determination of amino acid content of radiated samples. In previous studies the assumption has been made that one *ionization cluster* occurred within the confines of a single molecule and that each 100 eV produced an average of $1^1/_3$ ion clusters (75 eV/cluster). HUTCHINSON and POLLARD[9] have



Radiation inactivation of lysine vasopressin. Data from experimental series A. Each dot represents the mean of data from a single planchet. The dark line represents the calculated slope and the shaded area encompasses the 95% confidence limits of the *slope*.

Amino acid analysis of radiated lysine vasopressin

Radiation dose (rads)	Zero	$2.5 \cdot 10^8$		$10.0 \cdot 10^8$	
Amino acid	μmoles	μmoles	% of non-radiated	μmoles	% of non-radiated
Lysine	0.66	0.60	90	0.49	74
Ammonia	1.73	1.68	97	1.95	112
Aspartic acid	0.68	0.50	74	0.57	84
Glutamic acid	0.66	0.50	76	0.58	88
Proline	0.66	0.57	86	0.60	90
Glycine	0.68	0.48	71	0.53	77
Cystine	0.55	0.32	58	0.18	33
Tyrosine	0.56	0.43	77	0.41	73
Phenylalanine	0.62	0.50	81	0.48	77

Results are expressed as μmoles of recovered amino acid and % of non-radiated controls.

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[9] F. HUTCHINSON and E. POLLARD, in *Mechanisms in RadioBiology* (Eds., M. ERRERA and A. FORSSBERG; Academic Press, New York 1961).

noted that there is good agreement between known molecular weights and molecular weights estimated by radiation inactivation over a considerable range of weights, but that for molecules smaller than 10.000 the weight is often over-estimated. In the instances when the ionization *cluster* was considered to be the inactivating event for LVP, the estimated molecular weight was considerably greater than the known weight of 1078[8]. However, previous studies[10–12] have indicated that certain procedures may cause polymerization. It is possible, therefore, that radiation may have caused polymerization. The fact that the loss of biological activity follows an exponential curve, however, suggests that a homogeneous population of molecules was present. If one ionizing event destroyed a polymer and the molecules existed in numerous degrees of polymerization, then significant deviations from linearity would be expected. It therefore appears most likely that the inactivating event is a single ionization in this instance and that each ionization occurring within the confines of a LVP molecule destroys its biological activity.

In a previous study of adrenocorticotropin (ACTH)[4], it was observed that radiation in air resulted in loss of activity at a rate compatible with a much larger molecule than the rate of loss in a reduced argon atmosphere. ACTH may be inactivated by exposure to ozone alone, so that the increased loss of activity in air was felt to be summation of the effects of radiation and ozone inactivation (ozone is formed when ionizing particles pass through oxygen). For this reason studies were performed in the absence of oxygen for ACTH and, also to exclude this possibility, for LVP. The rate of inactivation of LVP also appeared slightly more rapid in air than in argon.

The data reported demonstrate actual destruction of amino acid residues. Interestingly, even though cystine was greatly decomposed, cysteic acid was not detected. Previous studies[13–15] on purified human and bovine fibrinogen have shown that radiation results in fragmentation of the molecule. The energy released by an ionizing event is relatively large (24 eV) in relation to the energy of chemical bonds[9] and thus it is not surprising that rupture of such bonds occurs.

Conclusions. The biological activity of lysine vasopressin is destroyed by exposure to ionizing radiation. Using the conventional target theory approach and assuming each ionization cluster is the inactivating event, estimations of molecular weight are considerably higher than actual molecular weight. If it is assumed that each ionizing event is also an inactivating event, estimates of molecular weight are in agreement with the known molecular weight. Destruction of amino acids occurred during the process of radiation.

Résumé. De la lysine vasopressine de haute pureté a été irradiée à l'air et dans une atmosphère d'argon sous une pression réduite à l'aide de doses progressives d'électrons monoénergétiques de 2 MeV. La vaso-constriction a été mesurée chez le rat et cette activité a diminué d'une manière exponentielle en fonction de la dose d'irradiation administrée. En utilisant la théorie anticathode de LEA, le poids moléculaire a été estimé à partir du taux de perte de l'activité. En supposant que chaque *ionisation* effective avait lieu à l'intérieur d'une molécule, le poids moléculaire estimé à partir des valeurs obtenues dans l'air a été de $1{,}1 \cdot 10$ (95% se situant de $9{,}0 \cdot 10^2$ à $1{,}3 \cdot 10^3$), et à partir des valeurs obtenues dans l'argon, il a été de $7{,}1 \cdot 10^2$ ($5{,}3$–$9{,}0 \cdot 10^3$). En supposant que chaque *faisceau d'ionisation* prenait place à l'intérieur d'une molécule, le poids moléculaire estimé était de $3{,}0 \cdot 10^3$. Des analyses d'acides aminés de la lysine vasopressine irradiée à $2{,}5 \cdot 10^8$ et à $10{,}0 \cdot 10^8$ rads ont été faites et comparées à celles effectuées sur l'hormone non traitée. Aux deux dosages d'irradiation, la destruction des acides aminés a été observée. La cystine a été l'acide aminé qui a été détruit dans la plus grande proportion; plus des $^2/_3$ détruits à la plus grande dose d'irradiation étudiée. On a donc conclu que dans le but de faire des estimations des poids moléculaires des polypeptides aussi petites que la vasopressine, on devrait faire des suppositions toutes différentes de celles qui avaient été conventionnellement utilisées pour les polypeptides plus grandes et pour les protéines. En plus de la fragmentation des protéines, l'irradiation semble provoquer la destruction des acides aminés particuliers.

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CORRIGENDUM

W. H. STAFFORD and J. P. WARD: *The Occurrence of 4-Ethyl and 2-5-Dimethylazulene in Cracking Column Products*, Experientia 22, fasc. 2, p. 87 (1966). On page 88, line 14 reads correctly as follows: '(found 11.9% N)[9]'. Footnote 7 reads as follows: 'The TNB complex has the same m.p. as that of 1, 4-dimethyl-8-isopropylazulene TNB complex[15].'. Footnote 9: '**cf.** N analysis of TNB complex of III'.