The Behavior of Copper Complex Forming Hydroxyphenazine in Normal and Leukemic Mice

Biological properties of chemical substances extracted from *Pseudomonas aeruginosa* have been under constant investigation since 1888[1]. Of interest is a fraction of *Pseudomonas aeruginosa* culture extractions that forms characteristic fibers if brought in contact with traces of copper[2]. This fraction was identified by Moos and ROWEN[3] as α-hydroxyphenazine, with tentative molecular weight[4] of 846·35 and ratio of copper to hydroxyphenazine (HP)[5] approximately 1:4. Studies concerning localization effects of these fibers in tissues of normal and leukemic mice are continuing, since these could be involved in molecular formation of tumors, and tumor-like strata. Liver and kidney contain maximum activity when animals are injected with Cu^{64}; however, similar studies in leukemic mice, to our knowledge, do not exist in the literature. To date, data have been obtained concerning changes in mice given Cu^{64} + hydroxyphenazine, and it is the purpose herein to summarize these studies.

Materials and Methods. Attempts were made to bring hydroxyphenazine into physiologically compatible solution from α-hydroxyphenazine prepared synthetically[6,7] by the Reaction Products Company of Painesville, Ohio, since yields from bacterial cultures were insufficient. Hydroxyphenazine in mineral oil allowed administration in amounts up to 10 mg/ml for use in tracer experiments and toxicity studies, and phenazine precipitated from EtOH was also used for toxicity studies. Amounts up to 1·0 ml of mineral oil containing 10 mg of hydroxyphenazine had no toxic effects on animals injected subcutaneously. Intravenous injections of saline-phenazine solutions were tolerated with any amount given to mice weighing 25–35 g. Cu^{64} (Abbott Laboratories; half-life 12·8 h[8]), was employed for tracer studies after finding that 0·1 mg of Cu citrate (pH 7·0, buffered) was not lethal for mice. Cu^{64} citrate activity was approximately 1–2 mc/ml at time of arrival and the solution was diluted with 0·036% sodium citrate to one-tenth this value, giving 0·2 mg Cu/ml with an activity of 100–200 μc/ml. Not over 0·5 ml (50–100 μc) was used for injection. Tracer experiments were performed on groups of 6–8 month old Carworth Farms normal (CFW) and leukemic (AKR) mice subdivided and treated as follows: (a) Cu^{64} injected intramuscularly (tail); (b) Cu^{64} injected intravenously (tail); (c) Cu^{64} injected intramuscularly, and 5·0 mg hydroxyphenazine in 0·5 ml oil given subcutaneously; (d) Cu^{64} injected intravenously, and hydroxyphenazine in oil given subcutaneously.

Mice were sacrificed 1 h after injections of Cu^{64}, or of the Cu^{64} + hydroxyphenazine, and the various organs removed, weighed, and evaporated to dryness in 10 ml of 90% formic acid over a steam bath. Dried organs were resuspended in 10 ml formic acid, and 1 ml aliquots of each suspension (in triplicate) was counted in a Nancy-Wood well counter, and the counts corrected for decay time, organ weight, and total animal weight.

Two long term survival studies on AKR mice (7–8-months old) were done to determine the effects of subcutaneous injections of Cu or HP, and Cu + HP. Leukemic mice in these studies were divided into four groups containing 10 animals each and injected as follows: (a) Copper citrate (0·1 mg Cu) subcutaneously; (b) Copper + hydroxyphenazine (0·1 mg Cu and 5·0 mg HP) subcutaneously; (c) Hydroxyphenazine (5·0 mg HP) subcutaneously; (d) Controls injected subcutaneously with sodium citrate (0·5 ml, 0·036%).

In the first survival study they were injected on the 2nd, 16th, 36th, 54th, and 126th day after arrival. In the second study they were injected on the 5th, 19th, 33rd, and 56th day after arrival. The animals were weighed weekly and the deviations in average weights of each animal group expressed as % change. Wright's smears were made for differential blood studies (tail-veins) on the 2nd, 20th, 36th, 54th, and 97th days after respective injections with Cu, Cu + HP, HP, and sodium citrate. Differential counts obtained during the duration (97 days) of survival and weight experiments were tested by Student's '*t*' test for significant differences.

Result. – Livers of all intramuscularly injected mice, and kidneys of all intravenously injected (Cu^{64} or Cu^{64} + HP) mice contained highest activity, and these organs were considered as having maximum (100%) activity. Smallest amount of Cu^{64} was always found in brain; stomach and duodenum-jejunum were relatively active; urine always contained large amounts of active material. Variation in some organs can be detected, and is attributed to the various diffusion rates from the site injected. Cu^{64} is excreted to a considerable extent within 1 h after intravenous injection. In contrast, subcutaneous injection of hydroxyphenazine in oil (1–20 h before injecting Cu^{64}) does not cause marked changes in organ distribution of Cu^{64} activity.

Normal and leukemic mice injected intramuscularly with Cu^{64}, and immediately injected with hydroxyphenazine subcutaneously exhibit no significant difference in organ activities; the sole exception being lung tissue. Intramuscular injection of Cu^{64} alone gave no significant difference in organ distribution, spleen being the exception in this series, instead of lung. However, quantitative comparisons between Cu^{64} injected alone, and Cu^{64} + α-hydroxyphenazine into leukemic mice, shows greater activity in spleen, lungs, and heart of leukemic mice receiving Cu^{64} + hydroxyphenazine. Both survival experiments gave good agreement, with the sole exception of the Cu + hydroxyphenazine treated mice. The short survival period of leukemic mice receiving Cu + hydroxyphenazine in the first survival study, and longer survival of mice in the second study receiving similar treatment, cannot be explained at present. Survival patterns of hydroxyphenazine and control (sodium citrate) injected mice gave no differences in the action of hydroxyphenazine or sodium citrate. Copper injections alone were of benefit, since lethality for both experiments was considerably lower than in mice receiving other treatment.

Leukemic mice bled on the 2nd, 20th, 36th, 54th, and 97th days following subcutaneous injections of Cu alone, Cu + hydroxyphenazine, hydroxyphenazine, and sodium citrate (control) showed no regular trend or pattern by '*t*' test over 97 days of treatment. Although Cu + HP and HP alone exerted more marked effect upon stimulation of PMN production than did Cu alone, or sodium

[1] C. BOUCHARD, C. R. Acad. Sci., Paris *108*, 713 (1889). – R. EMMERICH and O. LOW, Zbl. Bakt. *26*, 237 (1899).

[2] W. S. Moos, J. Bact. *62*, 767 (1951).

[3] W. S. Moos and J. W. ROWEN, Arch. Biochem. Biophys. *43*, 88 (1953).

[4] Acknowledgement is made to: W. C. ALFORD, Analytical Chemist, National Institutes of Health, Bethesda, Md., for the molecular weight studies.

[5] α-hydroxyphenazine is referred to in the Text and Tables as HP.

[6] H. MCILWAIN, J. chem. Soc. *1937*, 1704.

[7] H. ADKINS, Org. Synth. *26*, 86 (1946).

[8] J. MATTAUCH, *Nuclear Physics Tables* (Interscience Publ., New York 1946).

citrate, this could be due to chance. Lymphocyte production appeared depressed with Cu, Cu + HP, and HP in contrast to sodium citrate, but this was not significant. No significant tendency appeared for all the injected groups, even when compared to uninjected leukemic controls.

Injection of Cu^{64} into normal and leukemic mice by various routes, alone, or in combination with α-hydroxyphenazine, allowed us to evaluate the effects of HP and Cu on normal or leukemic mice. α-hydroxyphenazine was non-lethal in doses over 10 mg when injected into mice. Cu^{64} in combination with α-hydroxyphenazine gave twofold increases of activity in spleen, lung, and heart of leukemic mice. Such results were not obtained with normal mice receiving the combined injections, or in leukemic mice injected with Cu^{64} alone. The means whereby copper affects survival times when combined with hydroxyphenazine is not yet clear, since the blood picture is not significantly altered. Although survival time was prolonged in leukemic mice given copper alone, real depression or recovery from leukemic status could not be achieved by Cu + HP or α-hydroxyphenazine alone.

Fractionation by JACOBS[9] of *Pseudomonas aeruginosa* yielded a polysaccharide capable of inducing hemorrhage and necrosis to Sarcoma 37 of tumor-bearing mice. This effect was not induced by α-hydroxyphenazine in our normal or leukemic mice.

We are indebted to Mr. M. QUARLES and Mr. W. HENDERSON, Department of Radiology, University of Illinois, College of Medicine, for technical assistance.

W. S. Moos and H. C. MASON*

Department of Radiology, College of Medicine, University of Illinois and Illinois State Psychopathic Institute, May 12, 1959.

Zusammenfassung

Die Reaktion und die Ablagerungsstellen von Hydroxyphenazin und Kupfer wurden in gesunden und leukämischen Mäusen untersucht.

* Present address: Systems Management Office, Space Medicine Branch, Boeing Airplane Company, Seattle (Wash.).

[9] F. A. JACOBS, Cancer Res. *10*, 227 (1950).

Informations - Informationen - Informazioni - Notes

THEORIA

Zum physiologischen Potenzbegriff

(am Beispiel der Herzautomatie)

Vergleichend physiologische Untersuchungen über die Aktivität der Venenherzen der Chiroptera [1–3], Helixherzen[4], embryonalen Hühnchenherzen[5], aktivpulsierenden Armschirmhautgefässen der Cephalopoden[6,7], sowie entsprechende Untersuchungen meines Schülers HUGGEL[8] an embryonalen Fischherzen, verfolgten unter anderem die nähere Bestimmung der Gefäss- bzw. Herzpotenzen aus ihren Entwicklungsmöglichkeiten und ihrem Anpassungsvermögen. Mit der Erweiterung des experimentellen Erfahrungsbereichs schien es mir geboten, die gebräuchlichen Einzelbegriffe wie «Herzpotenz», «Herzautomatie» inhaltlich zu überprüfen und sie gegebenenfalls durch Umbildung und Differenzierung auf den gegenwärtigen Stand der Forschung zu bringen.

In der Überzeugung, dass die Arbeit am Begriffsapparat mit der Empirie Schritt halten sollte, bestärkte mich der 1937 in den Acta Biotheoretica erschienene, ausserordentlich anregende Aufsatz von RAVEN, *Der Potenzbegriff in der Entwicklungsmechanik*[9]. Darin verfolgt RAVEN das Ziel, den entwicklungsphysiologischen Potenzbegriff, wie er ursprünglich von DRIESCH in die «Entwicklungsmechanik» eingeführt worden ist, den «Bedürfnissen des Augenblicks» anzupassen, überzeugt, dass die Umbildung des Begriffes «keineswegs beendet» sei.

Die Frage, ob eine weitere Auflösung einen physiologischen Potenzbegriff auf späterer Entwicklungsstufe der Forschung entbehrlich machen wird, braucht uns im Augenblick, wo er noch ein fester Bestandteil unseres Begriffsapparates ist, nicht zu bedrängen. Wenn wir auch – wohl mit den meisten experimentell Arbeitenden – der Auffassung sind, dass geläufige physiologische Begriffe wie «Reaktivität», «Spontaneität», «Automatiepotenz» lediglich Ausdruck sind für zur Zeit noch unübersehbare «Faktoren-Konstellationen», so meinen wir doch, dass eine Begriffsweiterbildung der aktuellen Faktorenanalyse in allen Gebieten der Physiologie nach Möglichkeit parallel zu laufen oder wenigstens von Zeit zu Zeit «nachzuziehen» habe[10].

RAVEN selber hat bekanntlich nur den entwicklungsphysiologischen Potenzbegriff, die sogenannte «Differenzierungspotenz», untersuchen wollen und dabei klar gemacht, dass dieselbe nicht bloss als die Summe von Möglichkeiten des Keimmaterials aufzufassen sei, sondern als zusammengesetzt aus zwei Grundfähigkeiten des Substrates: 1. in geeignetem Milieu «spontan» bestimmte Differenzierungen hervorzubringen, 2. auf gewisse spezifische «Induktionswirkungen» mit bestimmt gerichteten Differenzierungen zu reagieren. Weiter, dass diese Entwicklungsfähigkeiten jeweils von ganz bestimmter Stärke sind, so dass die gesamte Differenzierungspotenz als eine «Stufenleiter von Differenzierungsfähigkeiten» aufgefasst werden kann, bei der Reaktionsfähigkeiten und Differenzierungstendenzen fliessend ineinander übergehen. RAVEN kommt dabei zur offenbar fundamentalen Einsicht, dass auch jede entwicklungsphysiologische Teilpotenz stets aus Reaktionsvermögen auf induzierende Reize und aus Eigentendenzen des Keimmaterials oder aus «passiver» und «aktiver» Potenz besteht, die somit unvermittelt ineinander übergehen. In Abhängigkeit von ihrer Intensität verschiebt sich zum Beispiel die Differenzierungspotenz auf der Stufenleiter «nach unten», wenn sie schwächer geworden und bei Unterschreiten der «Schwelle» in den Bereich der blossen Reaktionsfähigkeiten «herabsinkt», oder «nach oben», wenn sie potenziert die «Schwelle» über-

[1] H. MISLIN, Helv. physiol. Acta *5*, C 3 (1947).
[2] H. MISLIN, Exper. *10*, 28 (1948).
[3] H. MISLIN, Helv. physiol. Acta *17*, C 27 (1959).
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[5] H. MISLIN, Verh. schweiz. naturf. Ges. *129*, 153 (1948).
[6] H. MISLIN und M. KAUFMANN, Rev. suisse Zool. *55*, 267 (1948).
[7] H. MISLIN, Exper. *6*, 467 (1950).
[8] HJ. HUGGEL, Z. vgl. Physiol. *42*, 63 (1959).
[9] CHR. P. RAVEN, Acta biotheoretica *4*, 56 (1937).

[10] Was hier für unser Spezialgebiet gefordert wird, gilt natürlich auch für alle anderen naturwissenschaftlichen Disziplinen und ist nur durch die Schaffung einer vergleichenden Terminologie, einer *Terminologia Comparata*, zu leisten.