Biochemical Pharmacology, Vol. 32, No. 1, pp. 79–84, 1983.
Printed in Great Britain.

0006-2952/83/010079-06 $03.00/0

INFLUENCE OF pH ON THE CYTOTOXIC ACTIVITY OF CHLORAMBUCIL*

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(*Received* 4 *March* 1982; *accepted* 19 *May* 1982)

Abstract—The cytotoxic activity of chlorambucil as a function of pH was investigated in P388 tumor cells growing in static suspension culture. A decrease in extracellular pH from 7.8 to 7.2 was associated with a decrease in intracellular pH from 7.92 to 7.55. The cytotoxic potency of chlorambucil increased as the extracellular pH decreased; IC_{99} values were 20 and 60 μM when the extracellular pH was 7.2 and 7.8 respectively. Covalent binding to cellular macromolecules was about 1.9 times greater at pH 7.2 relative to that at pH 7.8. These results suggest that pH may be an important determinant of the oncotoxic specificity of chlorambucil, and that the cytotoxic activity of this agent could be selectively directed toward tumor cells by the selective manipulation of intracellular and extracellular pH. A potential influence of intracellular and extracellular pH on cytotoxic, mutagenic, carcinogenic, and teratogenic potencies of other chemicals is also suggested. Additionally, these investigations demonstrate the importance of carefully controlling pH throughout the drug exposure period when evaluating the relative potency of potential cytotoxic, mutagenic, carcinogenic, and teratogenic agents in cell or organ culture.

Chlorambucil is a nitrogen mustard analog with some clinical utility in the treatment of certain neoplasms. It is believed to exert its cytotoxic effect as a consequence of an intracellular action and to rapidly gain access to the intracellular target site by simple passive diffusion [1, 2]. Chlorambucil contains two ionizable groups, viz. a carboxylic acid (pK_a 5.8) and a tertiary nitrogen (pK_a 1.3) moiety [3]. The amount of a drug that enters cells by simple passive diffusion should be a function of the pK_a of its ionizable groups and the intracellular and extracellular pH since only non-ionized species readily cross biological membranes by this mechanism. Changes in intracellular pH need not be accompanied by changes of an equal magnitude in the extracellular pH [4]. In that event, changes in the amount of chlorambucil uptake and, hence, in the amount of cell-kill should be observed. This hypothesis was tested in the present investigation since it suggests a strategy potentially exploitable in the treatment of cancer with drugs.

MATERIALS AND METHODS

Materials. [Ring-U-^{14}C]chlorambucil (6.1 mCi/mmole) and [^{3}H]inulin (1.8 Ci/mmole) were purchased from the Amersham Corp., Arlington Heights, IL. [Carboxyl-^{14}C]inulin (1.55 mCi/g), [^{14}C]DMO§ (49.9 mCi/mmole), [^{3}H]water (1 mCi/g), [^{14}C]toluene (3.69×10^5 dpm/ml) and [^{3}H]toluene (2.20×10^6 dpm/ml) were purchased from the New England Nuclear Corp., Boston, MA. Radioactivity was quantified in Aquasol-2 scintillation mixture with a Packard model 3375 Tri-Carb liquid scintillation spectrometer; [^{14}C]- and [^{3}H]toluene were used to determine counting efficiencies.

Chlorambucil (NSC 3088) and HEPES were purchased from the Sigma Chemical Co., St. Louis, MO. BES and HEPPS were purchased from Calbiochem, La Jolla, CA. Dihydroxychlorambucil [*N*-bis(dihydroxyethyl)phenylbutyric acid] was generated by placing chlorambucil in 0.025 N sodium hydroxide for 60 min at 60° [5].

Mouse P388 tumor cells, established in continuous static suspension culture, were grown at 37° in RPMI 1640 medium supplemented with 10% horse serum and were continuously gassed with humidified 5% CO_2 in air. The mean population doubling time was about 10 hr.

No volatile organic zwitterion buffers [6] were used to control the extracellular pH throughout the 60-min drug exposure period routinely employed in these studies. Biological membranes are poorly permeable to these buffers. The complete formulation, designated OBD/HS medium, contained 40 mM HEPES, 20 mM HEPPS, and 10 mM BES dissolved in Dulbecco's phosphate-buffered saline

* Supported by American Cancer Society Grant CH-106. A description of this investigation has appeared in abstract form [G. T. Brophy and N. E. Sladek, *Pharmacologist* **19**, 208 (1977)] and in a thesis [G. T. Brophy, *Ph. D. Thesis*, University of Minnesota (1979)] submitted by G. T. Brophy in 1979 to the Department of Pharmacology, University of Minnesota, Minneapolis, in partial fulfillment of the requirements for the Ph.D. degree.

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§ Abbreviations: [^{14}C]DMO, [2-^{14}C]5,5-dimethyloxazolidine-2,4-dione; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; BES, *N,N*-bis-(2-hydroxyethyl)-2-amino-ethanesulfonic acid; HEPPS, *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid; TCA, trichloroacetic acid; TLC, thin-layer chromatography; pH_i, intracellular pH; and pH_e, extracellular pH.

supplemented with 10% horse serum and phenol red (5 mg/l). The pH was adjusted to the desired value with NaOH or HCl. The pH of all media was determined with a Radiometer Copenhagen model 26 pH meter equipped with a Corning model 476050 semi-micro combination electrode. The pH meter was calibrated with pH 7.00 and 8.00 ± 0.02 buffer standards. All media were sterilized by passage through a 0.22 μm Millipore filter; sterility was verified by routine tests.

Estimation of pH_i. The methods of Poole, Butler and Waddell [4, 7] were used, with some modification and integration, to determine the pH_i of P388 cells. The method requires that the extracellular aqueous fluid volume and the distribution of DMO between the intracellular and extracellular water be determined. Inulin was used to determine the small extracellular aqueous fluid volume remaining after cells were removed from the bulk of that volume.

P388 cells in asynchronous exponential growth were harvested and suspended (1×10^7 cells/ml) in OBD/HS medium adjusted to the desired pH. The cells were incubated at 37° for 45 min and then [^{14}C]DMO, [^{14}C]inulin and [^{3}H]water were added to final concentrations of 0.05, 0.5 and 5.0 μCi/ml respectively. The cells were then incubated for an additional 15 min at 37°. Preliminary experiments established that the distribution of [^{14}C]DMO and [^{3}H]water reached equilibrium within 15 min [4, 8]. At the conclusion of the incubation period, the cell suspension was divided into several portions which were centrifuged at 2000 *g* for 40 sec to generate supernatant and cell pellet fractions. The pH and the total ^{3}H- and ^{14}C-contents of some of the supernatant fractions were then determined. The remaining supernatant fractions were acidified with 5 M monobasic sodium phosphate and extracted with 5 vol. of ethyl acetate–toluene (1:1, v/v). The ^{14}C-content of the organic phase, containing [^{14}C]DMO (> 96% extraction efficiency) and [^{14}C]inulin (< 0.02% extraction efficiency), was then determined. The cell pellet fractions were submitted to similar analyses. The total ^{3}H- and ^{14}C-contents of some of the pellet fractions were determined after solubilization in Soluene-350. The remaining pellet fractions were extracted as described above for the supernatant fractions, and the ^{14}C-content of the organic phase ([^{14}C]DMO) was determined. The equation derived by Poole *et al.* [4] was used to calculate the pH_i. DMO and inulin concentrations in the cell pellet and supernatant fractions were normalized by expressing them per unit [^{3}H]water present in the appropriate fraction.

Quantification of chlorambucil equivalents present in acid-soluble and acid-insoluble fractions obtained from tumor cells exposed to chlorambucil. Chlorambucil molar equivalents in the acid-soluble and acid-insoluble fractions obtained from cultured tumor cells exposed to chlorambucil were quantified by a modification of methods described by Hill *et al.* [2] and Goldenberg *et al.* [9]. P388 cells in asynchronous exponential growth were harvested and suspended (1×10^7 cells/ml) in OBD/HS medium containing [^{14}C]chlorambucil. Following incubation at 37°, portions of the cell suspension were removed and the tumor cells were rapidly pelleted by centrifugation at 2000 *g* for 40 sec; quantitative cell recovery was obtained. After the supernatant fractions were removed by aspiration, the pellet fractions were washed twice to remove any remaining extracellular radioactive material; this was accomplished by resuspending them in ice-cold Earle's balanced salt solution lacking calcium, magnesium or phenol red, and recentrifuging at 2000 *g* for 40 sec.

The washed pellet fractions were then extracted twice with 0.5 ml of cold 5% TCA; complete separation of the TCA-soluble and TCA-insoluble fractions was effected by centrifugation at 2000 *g* for 40 sec. TCA-soluble fractions were transferred without further treatment into scintillation vials. After digestion in 0.4 ml of 0.5 N NaOH at 45°, the acid-insoluble fractions were neutralized with glacial acetic acid and also transferred to scintillation vials.

Assay for antitumor activity. A back-extrapolation assay was used, essentially as previously described [10, 11], to quantify the antitumor activity of chlorambucil. Cultured P388 tumor cells in asynchronous exponential growth were harvested and suspended in OBD/HS medium at a final concentration of 1.5 to 2.5×10^5 cells/ml. Chlorambucil, dissolved in absolute ethanol, or ethanol alone, was then added. Exposure of tumor cells to the drug and/or solvent was for 1 hr at 37° in air. The final ethanol concentration of 0.4% was without antitumor activity. At the end of the exposure period, the cells were harvested by low-speed centrifugation, and the drug-containing medium was removed. The tumor cells were resuspended in fresh RPMI 1640 medium supplemented with 10% horse serum and grown in static suspension culture at 37° in an atmosphere of 5% CO_2–95% air. Cell concentrations were determined at zero time and at approximately 24-hr intervals thereafter by a particle counter. Further details describing the assay have been published [10–13]. Incubation of P388 cells in OBD/HS medium alone for 1 hr at pH values ranging from 7.2 to 7.8 was without cytotoxic effect. All values are the averages of duplicate cultures.

RESULTS

The intracellular pH of cultured P388 cells was determined at medium pH 7.22 and 7.78 (Table 1). A difference of 0.56 units in pH_e was associated with a difference of only 0.37 units in pH_i.

Since the distribution of chlorambucil between the extracellular and intracellular compartments of a closed cultured tumor cell system should be governed solely by the pK_a of the drug and the pH on either side of the cell membrane, the Henderson–Hasselbalch equation can be used to predict it. At the intracellular pH values given in Table 1, a pK_a of 5.8, and steady-state kinetics, and assuming that only the non-ionized chlorambucil crosses the cell membranes, the Henderson–Hasselbalch equation predicts that chlorambucil will concentrate in the intracellular compartment of cultured P388 cells regardless of whether pH_e is 7.22 or 7.78 but that the intracellular concentration of chlorambucil at pH_e 7.22 will be 1.5 times greater than that at pH_e 7.78. The greater intracellular concentration of chlorambucil at pH_e 7.22 should be accompanied by

Table 1. Intracellular pH of P388 cells placed in pH 7.2 or 7.8 culture medium*

pH_e	ΔpH_e	pH_i	ΔpH_i
7.78		7.92 ± 0.05·	
	0.56		0.37
7.22		7.55 ± 0.01†	

* P388 cells were suspended in OBD/HS medium adjusted to pH 7.8 or 7.2 and incubation was allowed to proceed at 37° for 45 min. At the end of this time, the medium pH was 7.78 or 7.22 respectively. [^{14}C]DMO, [^{14}C]inulin and [^{3}H]water were then added and the incubation was continued for an additional 15 min. At the conclusion of the incubation period, the cell suspensions were centrifuged, and the DMO, inulin and water contents of the resultant pellet and supernatant fractions were determined and used to calculate the intracellular pH as described in Materials and Methods. Each value is the mean ± S.E. of four determinations from two individual experiments.

† Significantly different from pH_i obtained at pH_e 7.78 ($P < 0.1$).

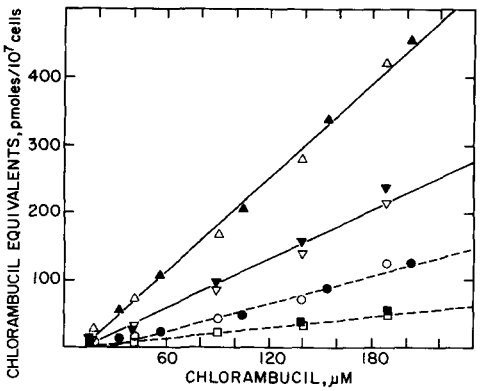

Fig. 2. Chlorambucil equivalents in acid-soluble and acid-insoluble fractions of cultured P388 cells exposed to various concentrations of chlorambucil for 1 hr at medium pH 7.2 and 7.8. P388 cells were exposed to [^{14}C]chlor ambucil (0.03 μCi/ml) for 1 hr at 37° in pH 7.2 or 7.8 OBD/HS medium. The data from two experiments are presented: (△,▲) pH 7.2, TCA-insoluble; (○,●) pH 7.2, TCA-soluble; (▽,▼) pH 7.8, TCA-insoluble; and (□,■) pH 7.8, TCA-soluble. Straight line functions were determined by linear regression analysis.

greater covalent binding of chlorambucil to cellular macromolecules and greater cell-kill.

The amount of radioactivity in the acid-insoluble fraction of tumor cells exposed to radiolabeled chlorambucil was assumed to reflect the amount of chlorambucil that was covalently bound to cellular macromolecules. In cultured P388 cells exposed to radiolabled chlorambucil at a pH_e of 7.2, the amount of radioactivity found in the acid-insoluble fraction was about 1.9 times greater than that found in this fraction when cells were exposed to the drug at a pH_e of 7.8 (Figs. 1–3). A time-dependent increase in the radioactivity of the acid-insoluble fraction was observed as would be expected if the radioactivity in this fraction reflected the covalent binding of chlorambucil (Fig. 1). Chlorambucil equivalents in the acid-insoluble fraction of cultured P388 cells increased as the concentration of chlorambucil in the medium increased (Fig. 2), and, as predicted, increased as the pH of that medium decreased (Figs. 1–3). Analysis of the data presented in Fig. 3 suggested that, at pH_e values of about 8.5 and higher, chlorambucil, at the concentrations employed in these experiments, would be without cytotoxic action.

Quantification of chlorambucil uptake into the acid-soluble fraction of cultured P388 cells was also attempted (Figs. 1–3). However, these measurements are of limited value because of the rapid rate (seconds) with which chlorambucil diffuses across the cell membrane [1, 2]; we estimate that up to 80% of the acid-soluble chlorambucil was lost from the cell during our washing procedure (data not presented). Nevertheless, the relative amounts of radioactivity found in the acid-soluble fractions of cultured P388 cells exposed to various concentrations of

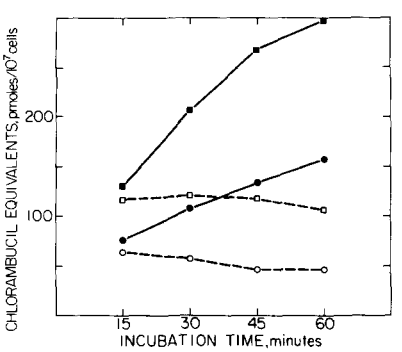

Fig. 1. Chlorambucil equivalents in acid-soluble and acid-insoluble fractions of cultured P388 cells exposed to chlorambucil at medium pH 7.2 and 7.8. P388 cells were exposed to 135 μM [^{14}C]chlorambucil (0.06 μCi/ml) at 37° in pH 7.2 or 7.8 OBD/HS medium. Key: (■) pH 7.2, TCA-insoluble; (□) pH 7.2, TCA-soluble; (●) pH 7.8, TCA-insoluble; and (○) pH 7.8, TCA-soluble.

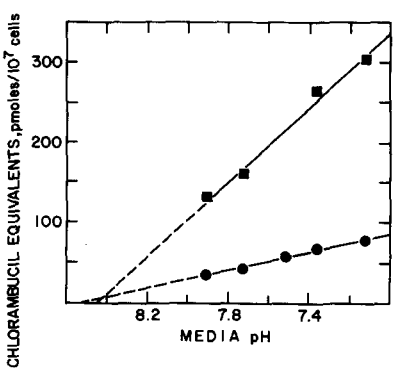

Fig. 3. Influence of medium pH on the content of chlorambucil equivalents in the acid-soluble and acid-insoluble fractions of cultured P388 cells exposed to chlorambucil for 1 hr. P388 cells were exposed to 115 μM [^{14}C]chlorambucil (0.06 μCi/ml) for 1 hr at 37° in OBD/HS medium adjusted to various pH values. Key: (●) TCA-soluble; and (■) TCA-insoluble. Straight line functions were determined by linear regression analysis.

radiolabeled chlorambucil for varying lengths of time were also about 1.9 times greater at a pH_e of 7.2 relative to the amounts found in these fractions when cells were exposed to the drug at a pH_e of 7.8.

To be sure that the relative values that we obtained for the acid-soluble radioactive material following incubation of P388 cells with [^{14}C]chlorambucil in pH 7.2 and pH 7.8 medium accurately reflected differences in intracellular free chlorambucil concentrations, the nature of the radioactive material was determined (Table 2). The percentage (22–24%) of the total intracellular ethanol-soluble radioactive material which co-chromatographed with authentic chlorambucil was independent of the pH of the medium employed during exposure to the drug.

Increased chlorambucil uptake and covalent binding with decreasing medium pH could not be due to differences in binding of the drug to horse serum proteins since binding was essentially pH independent in the pH range 7.2 to 8.0 (data not presented). The nature of the radioactive material present in the culture medium after incubation of chlorambucil at 37° for 1 hr was also examined as a possible explanation for the increased uptake of chlorambucil at the lower pH (Table 3). Virtually the same amounts of radioactivity co-chromatographed with authentic chlorambucil after a 60-min incubation in pH 7.8 and pH 7.2 culture medium supplemented with 10% horse serum. Thus, there were no differences in the binding, stability or reactivity of chlorambucil in the extracellular environment that would explain the enhanced uptake and covalent binding at the lower pH.

As predicted by the previous experiments, the cytotoxic potency of chlorambucil was also dependent on pH_e (Figs. 4 and 5). Tumor cell-kill was directly related to the concentration of chlorambucil in the medium and inversely related to the pH of the medium. The IC_{99} (concentration required to kill 99% of the cultured tumor cells) values were 20 and 60 μM when the pH_e values were 7.2 and 7.8 respectively (Fig. 4). As predicted by the data in Fig. 3, the data in Fig. 5 indicate that little or no cell-kill would be obtained if P388 tumor cells were exposed to chlorambucil in a medium with a pH greater than approximately 8.5.

Table 2. TLC of ethanol-soluble fractions obtained from tumor cells exposed to [^{14}C]chlorambucil*

	Percentage of total radioactivity R_f					
pH	0.0	0.15	0.16–0.47	0.48	0.55	0.69
7.8	7	2	5	24	24	37
7.2	5	4	7	22	26	36

* P388 cells in asynchronous exponential growth were harvested and suspended in pH 7.8 or 7.2 OBD/HS medium (5×10^7 cells/ml). [^{14}C]Chlorambucil, 325 μM (2 μCi/ml medium), was added to the cell suspension and incubation was allowed to proceed for 1 hr at 37°. Cells from 1.0-ml portions of the incubates were harvested and washed twice as described in Materials and Methods. Cold ethanol (0.5 ml) was added to the cell pellet, and extraction was allowed to proceed for 15 min. The resultant ethanol-soluble fractions were concentrated to near dryness under a stream of nitrogen and spotted on silica gel TLC plates. The plates were developed in methanol–chloroform (30:70, v/v), and the distribution of radioactive material was determined with the aid of a radiochromatogram scanner. The amount of radioactive material represented by each peak was expressed as a fraction of the total radioactive material present on the plate. The minimum detectable amount of ^{14}C was approximately 0.001 μCi. Authentic chlorambucil and dihydroxychlorambucil migrated at R_f 0.48 and 0.35 respectively.

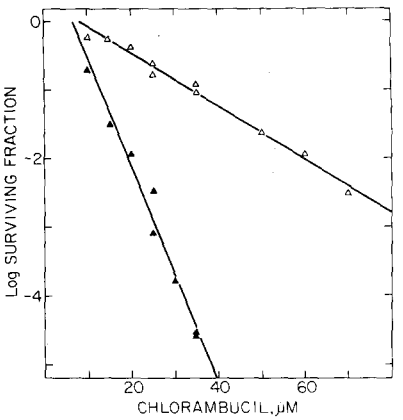

Fig. 4. Effect of chlorambucil on the growth of cultured P388 cells when exposure of tumor cells to chlorambucil was at pH 7.2 or 7.8. P388 tumor cells were exposed to chlorambucil for 1 hr at 37° in pH 7.8 (△) or pH 7.2 (▲) OBD/HS medium. The cells were then resuspended in drug-free RPMI 1640 culture medium supplemented with 10% horse serum, and the surviving fractions were determined by the back-extrapolation assay described in Materials and Methods. Straight line functions were determined by linear regression analysis.

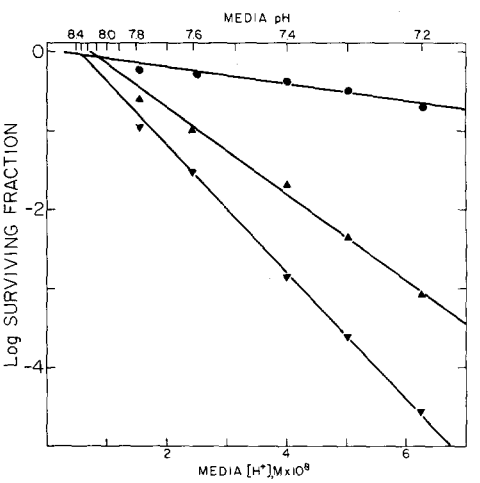

Fig. 5. Cytotoxic activity of chlorambucil toward cultured P388 cells: effect of chlorambucil concentration and medium pH during drug exposure. P388 cells were exposed to 10 μM (●), 25 μM (▲), or 35 μM (▼) chlorambucil for 1 hr at 37° in OBD/HS medium adjusted to the various pH values. Medium pH values were determined at the end of the incubation and did not differ from initial pH by more than 0.05 of a pH unit. The cells were then resuspended in drug-free RPMI 1640 culture medium supplemented with 10% horse serum, and surviving fractions were determined by the back-extrapolation assay described in Materials and Methods. Straight line functions were determined by linear regression analysis.

Table 3. TLC of the ethanol-soluble radioactive compounds obtained when [^{14}C]chlorambucil was incubated at 37° in OBD/HS medium*

Incubation time (min)	Percentage of total			
	pH 7.8		pH 7.2	
	$R_f = 0.0$	$R_f = 0.44$	$R_f = 0.0$	$R_f = 0.44$
0	0	100	0	100
15	9	91	8	92
30	15	85	9	91
45	18	82	13	87
60	25	75	21	79

* [^{14}C]Chlorambucil, 54 μM (0.08 μCi/ml), was added to pH 7.2 and pH 7.8 OBD/HS medium, and incubation was effected at 37° for 60 min. Fifty-microliter portions of the incubate were removed at various times during the incubation and extracted with cold ethanol. The resultant ethanol-soluble fractions, containing 97% of the total radioactive material present in the sample, were concentrated to near dryness and spotted on silica gel TLC plates. The plates were developed in methanol–chloroform (30:70, v/v) and scanned for the presence of radioactive material. The amount of radioactive material represented by each peak was expressed as a percentage of the total radioactive material present on the plate. The minimum detectable amount of ^{14}C was approximately 0.001 μCi. Authentic chlorambucil and dihydroxychlorambucil migrated at R_f 0.44 and 0.32 respectively.

The question of whether the increased cell-kill at pH 7.2 could be entirely accounted for by the increase in covalent binding to cellular macromolecules was addressed in Fig. 6. It is apparent that, for a given amount of chlorambucil equivalents in the TCA-insoluble cell fraction, more cell-kill was obtained at pH 7.2 than at pH 7.8.

DISCUSSION

Meaningful assessment of the relative cytotoxic, teratogenic, carcinogenic, or mutagenic activity of a drug in cell or organ culture may require that the pH of the medium by very carefully controlled during the time period when the cells are exposed to the drug. Data obtained in the present investigation demonstrate that the cytotoxic activity of chlorambucil is highly dependent on, and inversely related to, the pH of the culture medium; decreasing the pH of the medium from 7.8 to 7.2 increased the potency of chlorambucil approximately 3-fold. Changes in pH of this magnitude can easily occur during the drug exposure period when the relatively weak bicarbonate/phosphate buffer systems are employed, as is usually the case. Such changes are particularly likely to occur if the exposure period is lengthy and/or drugs that are, or can give rise to, acids or bases are added.

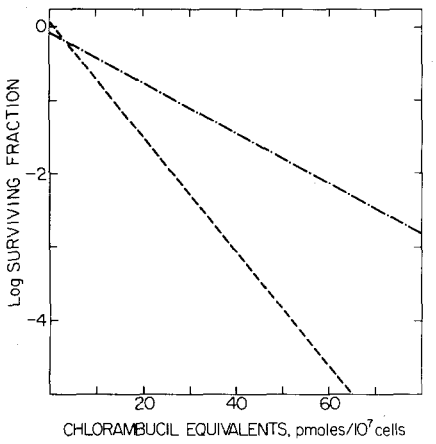

Fig. 6. Cytotoxic activity of chlorambucil as a function of chlorambucil equivalents in the TCA-insoluble fractions of cultured tumor cells. The equations defining the relations between the initial chlorambucil concentration in the culture medium and the amount of covalent binding to intracellular macromolecules (TCA-insoluble chlorambucil equivalents) (Fig. 2), and between the initial chlorambucil concentration in the culture medium and the log surviving fraction (Fig. 4), were simultaneously solved to obtain the following lines: (—·—) pH 7.8; and (———) pH 7.2.

The pH-dependent changes in the cytotoxic activity of chlorambucil can be explained, in part, by pH-dependent changes in its uptake and covalent binding. Since chlorambucil is ionized at physiological pH and enters cells by simple passive diffusion, pH-dependent changes in its uptake were as expected since changes in the pH of the medium were not accompanied by changes of equal magnitude in intracellular pH. The intracellular pH values obtained in the present investigation were somewhat higher than those obtained in other investigations [4, 8, 14, 15]; this may have been due to differences in tumor cell types or to differences in the buffering systems employed. In agreement with the present studies, Poole *et al.* [4] observed that, for a given decrease in extracellular pH, the magnitude of the decrease in intracellular pH was always less.

The pH-dependent differences in the amounts of covalent binding of the drug to macromolecules can not fully explain the associated pH-dependent changes in cell-kill. The values obtained for covalent binding undoubtedly represent averages of a population of values describing covalent binding to numerous macromolecules. A better correlation between covalent binding and cell-kill, as a function of medium pH, might have been obtained if covalent

binding to the critical target site, presumably DNA, had been determined. The amount of covalent binding to the critical target site is dependent upon the relative reactivities of that site and of alternative (non-critical) sites, the reactivity of the drug, and the amount of drug reaching the compartment that houses the critical site. Each of these may be pH-dependent. The buffering capacity and pH in any given compartment of the cell may differ substantially from the overall intracellular buffering capacity and pH, although we are not aware of any evidence supporting this possibility.

The basis for the limited oncotoxic specificity of chlorambucil is not known. In light of the results of the present investigation and the preceding considerations, it would seem that differences in intracellular pH may be important in that regard since the intracellular pH of some tumor cells may differ from that of normal cells [8, 16].

The results of the present investigation and the preceding considerations also indicate that the cytotoxic activity of chlorambucil could be selectively directed toward tumor cells by increasing the ratio:

$$[pH_i/pH_e]_{\text{tumor cell}}/[pH_i/pH_e]_{\text{normal cell}}$$

since there would be a relatively greater uptake of chlorambucil by the tumor cells as the ratio increased. An increase in this ratio would increase the oncotoxic specificity of not only chlorambucil, but of all antitumor agents that exert their cytotoxic action intracellularly, enter cells, at least in part, by simple passive diffusion, and are acids with favorable pK_a values. Increased uptake of bases with favorable pK_a values would be effected by a decrease in the ratio.

Selective alteration in tumor cell pH_i might be achieved by glucose administration since tumor cells may undergo a relatively high rate of anaerobic glycolysis leading to lactic acid accumulation [17, 18] although a high rate of anerobic glycosis may not be unique to tumor cells [19–22]. Indeed, some investigators have reported that the pH_i of tumor cells can be decreased by exposing them to high concentrations of glucose [14, 15]; others, however, report that the pH_i of tumor cells increases when glucose is introduced [8, 16]. Attempts to improve the therapeutic indices of alkylating agents by changing the pH of tumor tissue via glucose administration have thus far been largely unsuccessful [23–25]. Nonetheless, it would seem that selective manipulation of the tumor cell pH_i with the aim of improving the oncotoxic specificity of certain antitumor agents remains as a viable strategy.

The potential influence of pH_e and pH_i on the cytotoxic, teratogenic, carcinogenic, and mutagenic potencies of potentially hazardous chemicals should also be noted.

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