change in size or shape. Figures 9, *A* and *B*, are photomicrographs of the solid solution containing 20% griseofulvin. Before dissolution the particles appeared somewhat more elongated and irregular than the other samples considered. After exposure to the dissolution fluids (Fig. 9, *B*) the particles seem to be sintered and greatly reduced in size.

The photomicrographs serve to support graphically the quantitative findings of the dissolution rate studies. The most rapidly soluble forms of griseofulvin, *i.e.*, the solid solution and the eutectic mixture, manifest the most dramatic alterations in their crystalline nature after exposure to the dissolution medium.

The therapeutic advantages of the griseofulvin–succinic acid solid solution are presently under investigation in our laboratories. Extrapolation of the *in vitro* findings suggests the possibility that this form will provide more rapid and complete absorption of the drug, permit a reduction in dosage, and conceivably provide a more uniform therapeutic response. Of greater significance is the fact that this investigation has demonstrated a method of physical modification which could prove more important than micronization in enhancing the absorption and therapeutic effect of many water-insoluble drugs.

REFERENCES

(1) Fincher, J. H., Adams, J. G., and Beal, H. M., *J. Pharm. Sci.*, **54**, 704(1965).
(2) Atkinson, R. M., Bedford, C. B., Child, K. J., and Tomich, E. G., *Nature*, **193**, 588(1962).
(3) Goldberg, A. H., Gibaldi, M., and Kanig, J. L., *J. Pharm. Sci.*, **54**, 1145(1965).
(4) *Ibid.*, **55**, 482(1966).
(5) Levy, G., *Am. J. Pharm.*, **135**, 78(1963).
(6) Sekiguchi, K., and Obi, N., *Chem. Pharm. Bull. (Tokyo)*, **9**, 86(1961).
(7) Sekiguchi, K., Obi, N., and Useda, Y., *ibid.*, **12**, 134 (1964).
(8) Goldberg, A. H., Gibaldi, M., Kanig, J. L., and Shanker, J., *J. Pharm. Sci.*, **54**, 1722(1965).
(9) Guttman, D. E., and Brooke, D., *ibid.*, **52**, 941(1963).
(10) Gibaldi, M., unpublished data.
(11) May, D. R., and Kolthoff, I. M., *J. Phys. Colloid Chem.*, **52**, 836(1948).

The Antitumor Agent, 1,3-Bis(2-chloroethyl)-1-nitrosourea

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The new potent antitumor agent, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), is most stable at pH 4. In acid and in solutions above pH 7, it decomposes rapidly. In plasma, BCNU has a half-life of 20 min. *in vitro*, and less than 15 min. *in vivo*. Its alkylating action is not caused by the slow hydrolysis of the chlorine. BCNU is 80 per cent bound to human plasma protein at 0°. When administered intravenously to the dog, it enters the cerebrospinal fluid (CSF) readily and disappears speedily from the plasma and the CSF. The total amount of unchanged drug excreted in the urine in 4 hr. is less than 0.1 per cent of the dose. Heating at 43° for 5 hr. converts BCNU partly into 1,3-bis(2-chloroethyl)urea.

STUDIES OF the antitumor activity of derivatives of nitrosoguanidine (2) and nitrosourea (3) have led to the discovery of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a potent cancer chemotherapeutic agent of a novel type. BCNU is remarkable in that it is highly effective not only in intraperitoneal L1210 leukemia, but also in intracerebral L1210 leukemia (4), a distinct feature seldom found in most conventional agents. Unfortunately, clinical application of BCNU is limited because of unusual delayed toxicity in animals and man (5–7).

Chemically, although considered to be an alkylating agent, BCNU differs from typical derivatives of 2-chloroethylamine in having several reaction sites in addition to the carbon–chlorine bond which are potentially liable to attack by a variety of reagents under normal physiological conditions. Besides, the resultant transient chemical species may undergo further extensive biotransformations. These interesting considerations have prompted the authors to undertake a study on some of the chemical and pharmacological properties of BCNU. The present paper summarizes the results of these studies.

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This article is dedicated to the late Dr. E. K. Marshall, Jr., Professor Emeritus of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Md. The senior author gratefully acknowledges the counsel of Dr. Marshall on numerous occasions. The present work would have been impossible without the colorimetric method for sulfanilamide of Bratton and Marshall (1).

The authors thank Dr. Thomas Johnston for a gift of 1,3-bis(2-chloroethyl)urea and 1-(2-chloroethyl)-1-nitroso-3-cyclohexylurea.

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EXPERIMENTAL

BCNU.[1]—A sensitive and reproducible colorimetric method for the determination of BCNU has been described (8). The rate of decomposition was calculated by measuring the amount of unchanged BCNU after incubation with buffers or biological fluids. For the recovery of BCNU, buffers were treated the same way as biological fluids. Also, at pH above 8, the very fast rate of decomposition of BCNU necessitates the continuous direct measurement of absorbance at 230 mμ, the $\lambda_{max.}$ of BCNU. However, because of low molecular extinction (about 6,000), the direct measurement of absorbance is far less sensitive than the colorimetric method. In the range of 10–50 mcg./ml., a satisfactory linear relationship was found to exist between the concentration of BCNU and its absorbance at 230 mμ. However, the absorbance at 230 mμ assigned to $\pi \rightarrow \pi^*$ transition (9) could be attributed to the excitation of —N=N— as well as to that of —N=O. The decrease in absorbance at 230 mμ is a measurement of not only BCNU, but also its primary rearranged product (namely, the diazoester, see below). The colorimetric method is devoid of such ambiguities.

Determination of Chloride.—Microtitration of chloride was performed with an automatic Cotlove titrator (10). Nitrite anion derived from the nitroso group of BCNU was found not to interfere with the titration.

Protein Binding.—Protein binding was determined by ultrafiltration using the Toribara apparatus (11). The experiments were carried out at 0° because of the extreme instability of BCNU in plasma. A solution of BCNU in human plasma was prepared. An aliquot was kept at 0° throughout the duration of the experiment as a control. A measured volume of the remaining solution was placed in a cellulose sac (Visking Corp.) in the Toribara apparatus and spun at 2,200 r.p.m. for 2.5 hr. Further prolongation of centrifugation did not affect the results. After centrifugation, the BCNU in the control plasma, the plasma in the cellulose sac, and the ultrafiltrate were determined.

Plasma Level and Urinary Excretion in the Dog.—While under anesthesia with sodium pentobarbital,[2] female mongrel dogs weighing 10–16 Kg. received BCNU *via* the femoral vein, 10 mg./Kg., in a minimal volume of 50% ethanol. Samples of blood, 5 ml. each, were drawn from the opposite femoral artery with syringes wetted with heparin solution. Plasma was separated by centrifugation at 0°. Urine samples were collected by catheterization. Cerebrospinal fluid (CSF) (1.5 ml.) was sampled *via* the cisterna magna. All biological fluids were immediately frozen in a dry ice bath until analyzed. It is often desirable to prepare plasma and urine standards of the same animal as previously explained (8). Triplicate determinations were run whenever possible.

To achieve a constant plasma level, constant drug infusion was performed after an intravenous priming dose of 10 mg./Kg. The infusion solution was prepared by dissolving an appropriate amount of BCNU in a minimal volume of 95% ethanol and diluting with 0.2 *M* acetate buffer of pH 4.1. The latter was chosen because it is nearly isotonic and because BCNU is most stable (see below) at pH 4. The concentration of the infusion solution was such that the animal received 20 mg. of BCNU/Kg./hr. at a constant infusion rate of 0.5–1 ml./min.

Paper Chromatography.—Solutions of BCNU were applied to strips of Whatman No. 3 mm. paper and developed, ascending flow, by either 0.1 *M* sodium acetate buffer of pH 4.4 or petroleum ether of b.p. 100–115°. The instability of BCNU limits the solvent system to the above. Depending on BCNU concentration, the spots could be made visible on the dried paper by any of the following procedures. (*a*) Direct illumination with ultraviolet light of 254 mμ. (*b*) Spraying first with a solution of 50 mg. of sulfanilamide in 0.2 *N* hydrochloric acid, and 5 min. later with a solution of 0.3 Gm. of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 ml. of 95% *n*-butanol. (*c*) Spraying with a diphenylamine–palladium chloride reagent (12) consisting of 5 parts of 1.5% diphenylamine in ethanol and 1 part of 0.1% palladium chloride and 0.2% sodium chloride in water, followed immediately by irradiation with ultraviolet light of 254 mμ.

In acetate buffer system, BCNU gave an R_f value of 0.73 and in petroleum ether, 0.78.

Attempted Isolation of BCNU Metabolite from Dog Urine.—Following an intravenous injection of BCNU at 10 mg./Kg., the urine of a dog was collected for 3 hr. It was extracted 6 times with ether, using one-third its volume of ether each time. The combined ether extracts were dried over anhydrous magnesium sulfate and concentrated in an atmosphere of nitrogen at 0°. The presence of BCNU in the residual oil was confirmed by paper chromatography. However, other spots on the chromatogram were not identified. The oil resisted attempts at crystallization.

Thermal Decomposition of BCNU in Petroleum Ether.—BCNU, 500 mg., was dissolved in 50 ml. of petroleum ether (b.p. 30–60°) and refluxed at 43° (internal temperature) for 5 hr. The solvent was removed by vacuum distillation in a water bath at room temperature. After refrigeration overnight, the yellowish semisolid was triturated with ether and centrifuged. The supernatant was discarded and the residual solid was recrystallized twice from 30 ml. of ether containing 3 ml. of methanol. The fine colorless leaflets had an m.p. of 127–1:8°, undepressed by authentic 1,3-bis(2-chloroethyl)urea (13). The yield was 50 mg., 38%.

Anal.—Calcd. for $C_5H_{10}Cl_2N_2O$: C, 32.45; H, 5.45; Cl, 38.32; N, 15.14. Found: C, 32.52; H, 5.39; Cl, 37.97; N, 14.86.

RESULTS AND DISCUSSION

Stability of BCNU.—Table I summarizes the authors' results. The stability of BCNU is profoundly influenced by the pH of the solution. It is evident that BCNU is most stable in petroleum ether or in aqueous solution at pH 4. In acetate buffer of pH 4 at room temperature, its half-life exceeds 8 hr.

In strong acid (pH below 1), the rate of decomposition is too fast to measure. In fact, the ready

[1] Supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, N. J., through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md.

[2] Marketed as Nembutal by Abbott Laboratories, North Chicago, Ill.

TABLE I.—STABILITY OF BCNU: FIRST-ORDER RATE CONSTANTS AND HALF-LIFE PERIODS[a]

pH	Medium	Concn. of BCNU,[b] $mM \times 10^2$	Temp., °C.	$k \times 10^3$ min.$^{-1}$	$t_{1/2}$, min.
1	0.1 *N* HCl[c]	2.57	37	3.41	202
2.2	0.01 *M* K_2SO_4	2.34	37	1.66	416
2.2	0.01 *M* K_2SO_4	18.72	37	1.47	470
4.4	0.1 *M* NaOAc	2.57	37	1.35	511
5.4	Dist. water[c]	2.34	37	2.69	257
5.7	Urine[c]	2.34	37	2.48	278
6.0	0.1 *M* phosphate	2.34	37	2.20	314
6.8	0.1 *M* Tris	2.34	37	6.14	112
7.1	Plasma[c]	2.34	37	40.0	17
7.4	0.1 *M* barbiturate	2.34	37	13.3	52
7.4	0.1 *M* phosphate	2.34	37	13.3	52
7.5	0.005 *M* phosphate	18.72	37	20.4	34
7.8	Ringer's solution	2.34	37	26.3	26
8.0	0.1 *M* phosphate	18.72	25	4.51	152
8.0	0.01 *M* phosphate	18.72	25	4.89	141
8.2	0.1 *M* Tris	18.72	25	4.00	170
8.2	0.01 *M* Tris	18.72	25	4.70	147
8.2	0.001 *M* Tris	18.72	25	3.82	181
8.3	0.1 *M* NH_4HCO_3	18.72	25	6.29	110
8.3	0.01 *M* NH_4HCO_3	18.72	25	7.73	89
8.8	0.1 *M* borate	9.36	25	12.0	57
8.8	0.1 *M* borate	18.72	25	12.0	57
9.3	0.1 *M* Tris	14.04	25	29.5	23
	Petroleum ether[c]	14.04	50	0.94	730

[a] Average of three experiments. [b] Solutions more concentrated than 2.57×10^{-2} m*M* were studied by direct spectrophotometric measurement of absorbance at 230 mμ. [c] Unbuffered solutions.

liberation of nitrous acid by BCNU in strong acidic solution constitutes the basis for its colorimetric determination. Between pH 1 and pH 4, the decomposition is first order with respect to BCNU. Its relative stability at pH 1 (half-life longer than 3 hr.) agrees with the observation that BCNU is effective by mouth.

From pH 4.4 to pH 7.8, the decomposition still follows first-order kinetics; however, as explained below, the mechanism is probably not the same as in the more acidic media. The results of some typical experiments are graphically represented in Fig. 1. In the pH range of 4 to 9 the rate varies directly with pH,[3] but it is independent of the nature and concentration of the buffer. It is naturally affected by temperature: at room temperature (25°) the rate of decomposition is about 35% slower than at body temperature (37°). Also, at pH above 9.3, or even at pH 8, but at 37° instead of 25°, the decomposition of BCNU becomes complex kinetically, and the rate is not only very fast, but also no longer first order. Its half-life at pH 10 is not much more than 5 min.

In urine, BCNU is about as stable as in water of comparable pH. But with plasma, the case is entirely different. It decomposes at a rate 3 times faster than in phosphate buffer of pH 7.4, and its *in vitro* half-life in plasma is about 20 min. Although fast, the decay still obeys first-order kinetics, in distinct contrast with the *in vivo* situation. In the latter case, the decay of BCNU is not only somewhat faster, but follows complex kinetics because of biotransformation and excretion. In Ringer's solution, however, the decomposition is unremarkable. Clearly, plasma accelerates the destruction of BCNU *in vitro*.

[3] A rate expression could be empirically derived,

$$-\frac{d(\text{BCNU})}{dt} = k(\text{BCNU})/(\text{H}^+)^{0.3}$$

at 25° for the range pH 4 to pH 9.

The stability of BCNU was also studied in two organic solvents, namely, methanol and petroleum ether (b.p. 100–115°) by direct spectrophotometry. In the former at 50°, the decomposition of BCNU is rapid and the kinetics is complicated. On the

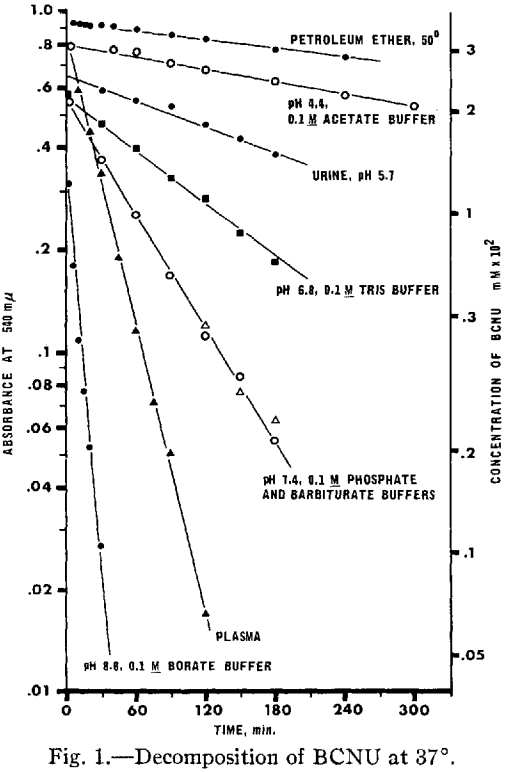


Fig. 1.—Decomposition of BCNU at 37°.

other hand, BCNU appears to be relatively stable in the latter even at 50°, and the decay obeys first-order law.

Alkaline Hydrolysis of BCNU.—It is difficult, perhaps impossible, to ascertain meaningfully the true rate of hydrolysis of the chloro- group in BCNU because of the extreme instability of BCNU in alkaline solution. However, since BCNU is generally considered an alkylating agent, we undertook a study of the rate of release of chloride ion by BCNU in order to compare it with other well-known alkylating agents. As expected, a semilog plot of either chloride liberated or fractional BCNU unhydrolyzed *versus* time fails to reveal any kinetic trend. The rate of hydrolysis is relatively slow; no more than 22% of the theoretical amount of chlorine was set free by incubation of BCNU at 25° for 90 min. in 0.1 *M* borate buffer of pH 9.8 at concentrations ranging from 0.187 m*M* (40 mcg./ml.) to 2.34 m*M* (500 mcg./ml.). At 37° in 0.1 *M* phosphate buffer of pH 7.4, a 2.34 m*M* solution of BCNU showed 31.6% of hydrolysis after more than 5 hr. The same solution exhibited 87% hydrolysis only after heating at 50° for 24 hr. As stated above, these rates could not really pertain to BCNU because under the experimental conditions BCNU has a half-life of about 5 min. to less than 1 hr. Obviously, in spite of the common biochemical effects (14) and cross resistance shared by BCNU and other alkylating agents (4, 15, 16), BCNU does not belong to the group of alkylating agents such as bis(2-chloroethyl)methylamine. If it is an alkylating agent at all, it must owe the alkylating action to one of its degradation products.

Protein Binding.—With a solution of 9.36×10^{-5} *M* of BCNU in human plasma, the results of a typical experiment are shown in Table II.

The percentage of BCNU bound to plasma protein is: (0.760–0.170)/0.760 × 100 or 78%, and the percentage recovery is: (0.760 × 4.5) + (0.170 × 0.5) × 100/(0.784 × 5.0) or 109%. On the basis of three experiments, the average extent of binding of BCNU with human plasma protein is about 80% at 0°.

The fraction of plasma protein involved in drug binding usually is albumin. Assuming an albumin concentration of 5×10^{-4} *M* in human plasma (17, 18), and also there is only one single binding site for BCNU per molecule of human plasma protein, the association constant, *K*, of the plasma protein–BCNU complex is easily estimated:

$$K = \frac{(P \cdot \text{BCNU})}{(P_f)(\text{BCNU})} = \frac{0.80 \times 9.36 \times 10^{-5}}{(5 \times 10^{-4} - 0.80 \times 9.36 \times 10^{-5})(0.20 \times 9.36 \times 10^{-5})}$$

$$= 9.4 \times 10^{3}\ M^{-1} \text{ at } 0°$$

which is comparatively small.

TABLE II.—RESULTS OF TYPICAL EXPERIMENT

	Absorbance at 540 mμ	Vol., ml.
Control	0.784	5.0
Inside	0.760	4.5
Ultrafiltrate	0.170	0.5

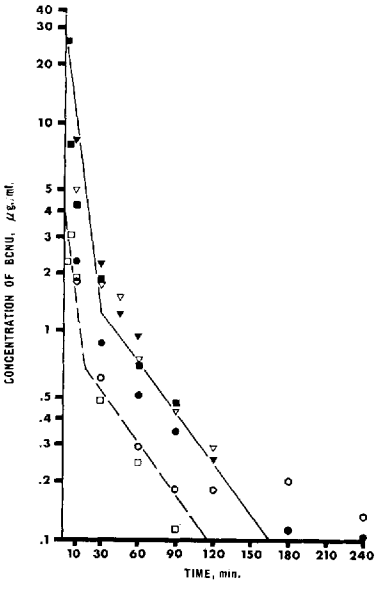


Fig. 2.—Plasma and CSF levels of BCNU in the dog. Key: ○, dog 1; □, dog 2; ▽, dog 3. (Solid symbols indicate plasma; open symbols indicate CSF.)

Plasma Level and Urinary Excretion in the Dog.—The rapid decline of BCNU in the plasma of the dog after a single intravenous injection is shown in Fig. 2. For the first 30 min. the fall of plasma levels in the three animals does not deviate too much from an exponential pattern and the half-life in plasma seems to be less than 15 min. Although protein binding by BCNU appears extensive, the association constant is relatively small. Consequently, even

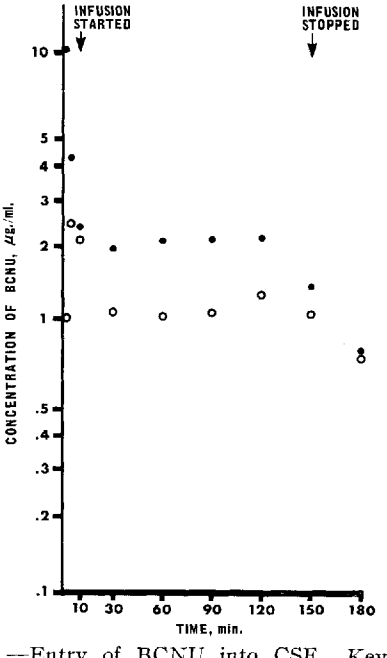
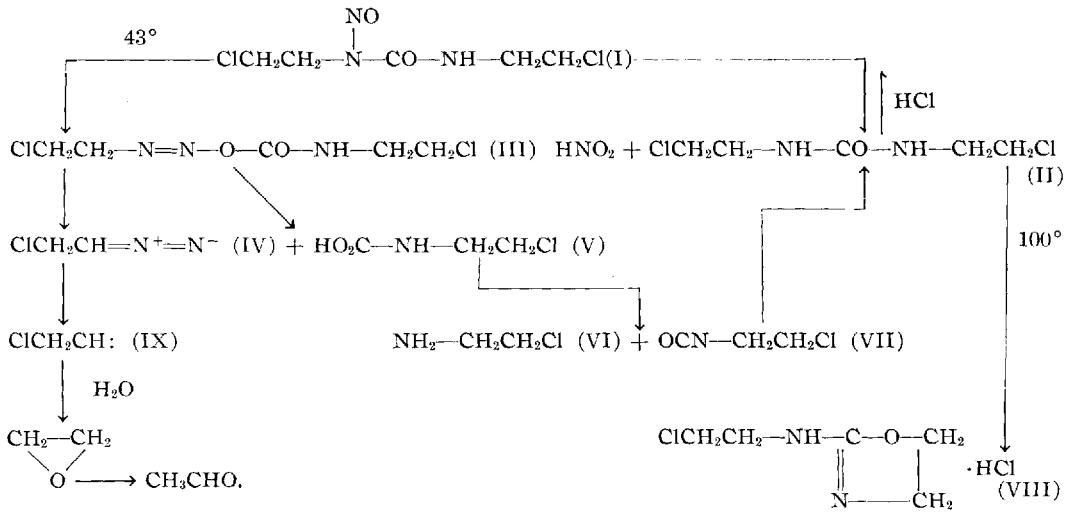


Fig. 3.—Entry of BCNU into CSF. Key: ●, plasma; ○, CSF.



Scheme I

though an increased proportion of drug would be bound as the total amount of BCNU decreases, this estimation of half-life in plasma is essentially correct (17). Extrapolation to zero time gives a zero time plasma level of about 30 mcg./ml. Since the dose of BCNU in these experiments was 10 mg./Kg., the volume of distribution is therefore 33% of body weight, in other words, somewhat larger than the volume of extracellular water. This is unexpected because BCNU, being highly soluble in lipids, appears to penetrate cellular membranes freely. In fact, BCNU makes its appearance in the CSF and the blood stream simultaneously. Furthermore, its concentration in the former falls at about the same rate as in the latter.

In view of the exceptional chemical reactivity of BCNU, it is necessary to maintain a steady plasma level by infusion before a meaningful estimate of the extent of entry into the CSF could be made. Figure 3 illustrates the results of such an experiment. After a constant plasma level of the drug has been achieved, the CSF level of BCNU reaches about 48% that of plasma (average of three experiments), confirming the screening data and clinical experience that BCNU readily penetrates the blood-brain barrier. At first sight this ratio appears to be higher than the percentage of free BCNU in plasma (20%). However, it should be recalled that protein binding was determined with human plasma at 0° and besides, the cellulose sac used is only a crude facsimile of cell membrane.

Ten minutes after a single intravenous injection, BCNU begins to appear in the urine. The excretion reaches a peak at about 2 hr. and then gradually tapers off until 3 hr. later when a detectable amount of the drug was present. Nevertheless, in no case is the total amount of unchanged BCNU excreted in 4 hr. in excess of 0.1% of the dose.

That the colorimetric method actually measures unchanged BCNU in plasma, CSF, and urine is supported by paper chromatography. When applied on Whatman No. 3 mm. paper and developed by the systems described above, the ether extracts of these biological fluids exhibit spots with R_f values identical to those obtained from ether extracts of the same fluid after the addition of authentic BCNU.

Doubtlessly BCNU undergoes extensive catabolism in the body since the percentage of unchanged BCNU recoverable from urine is so low. Although its biotransformation must await further studies, some indications could be gleaned from its thermal decomposition.

Thermal Decomposition of BCNU in Petroleum Ether.—The authors chose to undertake the study in a nonpolar solvent, namely, petroleum ether, so as to minimize secondary reactions. The isolation of 1,3-bis(2-chloroethyl)urea (II), after heating BCNU at 43° for 5 hr., suggests the postulated sequence of events in Scheme I.

The proposed 4-centered thermal rearrangement of BCNU (I), a nitrosamide, to the diazoester (III), is well known (19, 20). This is perhaps also the first step in the decay of BCNU in aqueous solutions above pH 4. The diazoester (III), incapable of but a fleeting existence, at once decomposes by an α-elimination analogous to the case of *N*-(*n*-butyl)-*N*-nitrosotrimethylacetamide (21) to give the diazoethane (IV) and the carbamic acid (V). The fate of the diazoethane (IV) cannot be defined by this experiment. However, the isolation of acetaldehyde from an aqueous solution of BCNU after refluxing (22) implies that the acetaldehyde originates from an analogous ephemeral intermediate, 2-chloroethyldiazoic acid, $ClCH_2CH_2N{=}NOH$, *via* ethylene oxide. Possibly, therefore, in this work the diazoethane (IV), on losing nitrogen, becomes transformed into the reactive carbene (IX) which also hydrolyzes to acetaldehyde. In aqueous solution, the intermediate steps would be similar.

It is not clear exactly how 1,3-bis(2-chloroethyl)-urea (II) is derived from the carbamic acid (V); the over-all reaction, nevertheless, must result from 2 moles of V through the loss of 1 mole each of water and carbon dioxide. The authors suggest 2-chloroethyl isocyanate (VII)[4] as a possible inter-

[4] Dr. R. E. Larson independently made the same suggestion that 2-chloroethyl isocyanate might be one of the intermediate biotransformation products of BCNU.

mediate not only because the addition of 2-chloroethylamine (VI) to the postulated 2-chloroethyl isocyanate (VII) explains very well the formation of 1,3-bis(2-chloroethyl)urea (II), but also because the delayed hepatoxicity of BCNU strongly resembles that of α-naphthyl isocyanate. In any event, the thermal decomposition of BCNU (I) to afford the original unnitrosated 1,3-bis(2-chloroethyl)urea (II) cannot be explained by the simple loss of a nitroso group directly from BCNU for the following reasons: first of all, such a homolysis is unprecedented; second, and most important, when 1-(2-chloroethyl)-1-nitroso-3-cyclohexylurea undergoes a like reaction in petroleum ether (b.p. 66°–75°) at 67° overnight, the product is 1,3-dicyclohexylurea [m.p. 224°, identical to an authentic sample (23) in every respect] and not 1-(2-chloroethyl)-3-cyclohexylurea, m.p. 130°–132° (24).

It has also been shown that 1,3-bis(2-chloroethyl)-urea (II) readily cyclizes in boiling water to 2-(2-chloroethylamino)-2-oxazoline (hydrochloride) (VIII) (25). However, we have no evidence that this has occurred at 43° in petroleum ether. Whether this is true *in vivo* remains to be seen.

SUMMARY

1. BCNU is most stable at pH 4. In acid as well as in aqueous solutions of pH greater than 7 it is shortlived. Its half-life in plasma is about 20 min. *in vitro* and less than 15 min. *in vivo*.

2. The release of Cl^- by hydrolysis of BCNU is too slow to account for its alkylating action.

3. BCNU is 80% bound to human plasma protein at 0°.

4. In the dog, after a single intravenous injection, BCNU enters the CSF immediately and then disappears rapidly from both the blood stream and the CSF. No more than 0.1% of unchanged drug is excreted in 4 hr. Nothing is known concerning its biotransformation *in vivo*.

5. Refluxing of BCNU at 43° in petroleum ether for 5 hr. converts it partly into 1,3-bis(2-chloroethyl)urea. The mechanism of this reaction is discussed.

REFERENCES

(1) Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, **128**, 537(1939).
(2) Skinner, W. A., *et al.*, *J. Med. Pharm Chem.*, **2**, 299 (1960).
(3) Johnston, T. P., McCaleb, G. S., and Montgomery, J. A., *J. Med. Chem.*, **6**, 669(1963).
(4) Schabel, F. M., Jr., *et al.*, *Cancer Res.*, **23**, 725(1963).
(5) Rall, D. P., Ben, M., and McCarthy, D. M., *Proc. Am. Assoc. Cancer Res.*, **4**, 55(1963).
(6) DeVita, V. T., *et al.*, *Cancer Res.*, **25**, 1876(1965).
(7) Larson, R. L., and Rall, D. P., *Pharmacologist*, **7**, 180 (1965).
(8) Loo, T. L., and Dion, R. L., *J. Pharm. Sci.*, **54**, 809 (1965).
(9) Layne, W. S., Jaffe, H. H., and Zimmer, H., *J. Am. Chem. Soc.*, **85**, 435(1963).
(10) Cotlove, E., Trantham, H. V., and Bowman, R. L., *J. Lab. Clin. Med.*, **51**, 461(1958).
(11) Toribara, T. Y., Terepke, A. R., and Dewey, P. A., *J. Clin. Invest.*, **36**, 738(1957).
(12) Preussmann, R., Daiber, D., and Hengy, H., *Nature*, **201**, 502(1964).
(13) Bestian, H., *Ann. Chem.*, **566**, 210(1950).
(14) Wheeler, G. P., and Bowden, B. J., *Cancer Res.*, **25**, 1770(1965).
(15) Pittillo, R. F., Narcates, A. J., and Burns, J., *ibid.*, **24**, 1228(1964).
(16) Skipper, H. E., and Schabel, F. M., Jr., *Cancer Chemotherapy Rept.*, **22**, 1(1962).
(17) Martin, B. K., *Nature*, **207**, 274, 959(1965).
(18) Goldstein, A., *Pharmacol. Rev.*, **1**, 102(1949).
(19) Huisgen, R., and Reimlinger, H., *Ann. Chem.*, **599**, 11(1956); and earlier papers.
(20) Heyns, K., and von Bebenburg, W., *ibid.*, **595**, 55 (1955).
(21) White, E. H., and Aufdermarsh, C. A., Jr., *J. Am. Chem. Soc.*, **83**, 1174(1961).
(22) Montgomery, J. A., personal communication.
(23) Skita, A., and Rolfes, H., *Chem. Ber.*, **53**, 1248(1920).
(24) French pat. 1,313,055(1962); through *Chem. Abstr.*, **59**, 7533*e*(1963).
(25) Kreling, M.-E., and McKay, A. F., *Can. J. Chem.*, **37**, 504(1959).

Retention, Excretion, and Distribution of 2,3,5-Triiodobenzoic Acid and Its Metabolites in the Rat

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The synthesis of 2(^{131}I),3,5-triiodobenzoic acid (TI*BA), starting with 2-amino-3,5-diiodobenzoic acid and sodium iodide (^{131}I) is described. The rate of excretion, biological half-life, and metabolites of this compound in rats were studied. Four metabolites and TIBA were found in the urine.

In soybeans, TIBA affects plant morphology and flowering response (1, 2). When properly used it increases bean production through a better utilization of photosynthate (3). Since soybeans are used for animal and human consumption, the question arises as to the environmental health hazards of TIBA and its metabolites.

TIBA was originally synthesized by Wheeler and Johns (4). Anthranilic acid was reacted with iodine monochloride and the resultant product diazotized and reacted with potassium iodide. The synthesis of TI*BA has been de-

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