were injured and embolization in both lasted for about the same periods of time. In the course of the experiments an interesting difference in the duration of embolization was observed depending on whether the rats were anaesthetized with sodium pentobarbital or ether instead of with urethane. The experiments which showed this difference were as follows:

Male and female albino rats of the Wistar strain, weighing 190–250 g, were anaesthetized and the skull and dura carefully removed from one cortex to expose an area measuring about 15 × 7 mm. This area was kept moist by a gentle flow of warmed physiological saline and the temperature of the cortical surface was maintained at about 30° C. The exposed vessels were observed under a Zeiss model II dissecting stereomicroscope at a magnification of 42 times. A vein, about 100μ in diameter, was injured by pinching it with fine ophthalmic forceps so that it bled for 10–40 sec (under ether anaesthesia the bleeding usually went on for 90 sec or even longer). White bodies began to form after 2–5 min and the time from the embolization of the first white body to that of the last was recorded. Several veins in each rat were injured and timed in this manner and the longest period of embolization was used for statistical comparisons.

Table 1 shows that injured cortical vessels produced embolizing white bodies for significantly longer periods of time under urethane anaesthesia than under anaesthesia with either sodium pentobarbital or ether. It seems, therefore, that urethane in anæsthetic concentrations greatly prolongs the production of platelet thrombi in injured vessels.

Table 1

Anaesthetic	No. of rats	Duration of embolization (min) Range	Mean	S.E.M.	P
Sodium pentobarbital	11	4–61	18·9 ±	5·79	0·001
Urethane	10	47–240+	106·7 ±	19·64	0·001
Ether	8	4–43·5	26·4 ±	4·91	

This observation is presumably related to the irritant and cytotoxic properties of urethane. Urethane has been used as a sclerosing agent in the treatment of varicose veins[6–10] and as a cytotoxic agent in the treatment of leukaemia and other malignancies[11–16]. Urethane increases capillary permeability[17,18], and it causes severe damage to portal veins and sinusoidal capillaries in rats[19]. These experiments suggest that urethane, in anaesthetic concentrations, somehow irritates vascular endothelium sufficiently to prolong the effects of mechanical trauma as indicated by the intravascular adhesion and aggregation of platelets.

G. V. R. Born
R. B. Philp

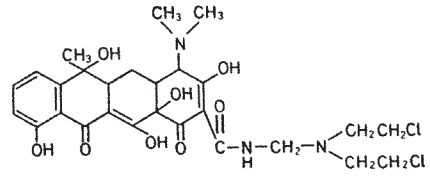
Department of Pharmacology,
Royal College of Surgeons,
Lincoln's Inn Fields,
London, W.C.2.

[1] Florey, H., *Brain*, **48**, 43 (1925).
[2] Honour, A. J., and Ross-Russell, R. W., *Brit. J. Exp. Path.*, **43**, 350 (1962).
[3] Honour, A. J., and Mitchell, J. R. A., *Nature*, **197**, 1019 (1963).
[4] Born, G. V. R., *Nature*, **202**, 95 (1964).
[5] Born, G. V. R., Honour, A. J., and Mitchell, J. R. A., *Nature*, **202**, 761 (1964).
[6] Barber, T. H. T., *Brit. Med. J.*, ii, 59 (1930).
[7] Bartlett, S., *Brit. Med. J.*, i, 153 (1929).
[8] Howard, N. J., Jackson, C. R., and Mahon, E. J., *Arch. Surg.*, **22**, 353 (1931).
[9] Payne, R. T., *Lancet*, ii, 313 (1929).
[10] Whelan, H. M., *J. Roy. Nav. Med. Serv.*, **15**, 203 (1929).
[11] Duston, jun., P., *Brit. J. Cancer*, **1**, 48 (1947).
[12] Haddow, A., and Sexton, W. A., *Nature*, **157**, 500 (1946).
[13] Kirschbaum, A., and Lu, C. S., *Proc. Soc. Exp. Biol. and Med.*, **65**, 62 (1947).
[14] Law, L. W., *Proc. U.S. Nat. Acad. Sci.*, **33**, 204 (1947).
[15] Paterson, E., Haddow, A., Ap Thomas, J., and Watkinson, J. M., *Lancet*, i, 677 (1946).
[16] Skipper, H. E., and Bryan, E. C. E., *J. Nat. Cancer Inst.*, **9**, 391 (1949).
[17] Krogh, A., and Harrop, G. A., *J. Physiol.*, **54**, 125 (1920–21).
[18] Landis E. M., *Amer. J. Physiol.*, **81**, 124 (1927).
[19] Dokjanski, L., and Rosin, A., *Amer. J. Path.*, **20**, 945 (1944).

Antitumour Activity of *N*-(β,β′-dichlorodiethylaminomethyl)-tetracycline, 'Tetracycline-mustard'

Localization of tetracycline in tumour tissue has been reported with both human and animal neoplasms[1]. Though the tetracycline class antibiotics have no antitumour effect by themselves, the possibility of using them as carriers of more potent agents seemed worthy of investigation. As a model system a nitrogen mustard (HN2) derivative was considered most practical because of simplicity of structure, availability of starting materials and general knowledge concerning the antitumour activity of mustard type alkylating agents.

Preparation of *N*-(β,β′-dichlorodiethylaminomethyl)-tetracycline was carried out according to the carboxamido-Einhorn derivative method of Gottstein, Minor and Cheney[2]. Fifty g of tetracycline base, 20·6 g of β,β′-dichlorodiethylamine hydrochloride (*nor* HN2), and 7 g of formaldehyde (14 ml. of 55 per cent methylformcel) were dissolved in 250 ml. of methanol. This solution was held at 22° C for 1·5 h and then added with rapid stirring to 3 l. of ethyl ether. The resulting precipitate was removed by filtration, washed with 300 ml. of ethyl ether and dried at 50° C *in vacuo* for 24 h. Fifty-five g of tetracycline-mustard Einhorn derivative (TCM) were obtained. This amorphous material was tested without further treatment since extensive hydrolysis was noted with various crystallization techniques. Analytical data for TCM as *N*-(β,β′-dichlorodiethylaminomethyl)-tetracycline were : theory—(for $C_{27}H_{33}N_3O_8Cl_2$) per cent chlorine, 11·85; per cent nitrogen, 7·02; antibacterial assay potency, 760 tetracycline units/mg; found—per cent chlorine, 11·6; per cent nitrogen, 6·3; assay potency 730 tetracycline units/mg. On the assumption of an Einhorn reaction a tentative structure is proposed here.



(1) *N*-(β,β′-dichlorodiethylaminomethyl)-tetracycline.
(2) Epithets: tetracycline mustard, TCM.

Tetracycline mustard was tested under the auspices of the Cancer Chemotherapy National Service Center at Hazleton Laboratories against the *in vivo* mouse tumours sarcoma 180 (*S*-180), adenocarcinoma 755 (*Ca*755) and leukæmia 1210 (*L*-1210). Inhibition of *Ca*755 was observed at doses of 80 and 60 mg/kg/day (intraperitoneal injections) whereas *S*-180 was not reproducibly inhibited. Significant prolongation of survival time of mice with *L*-1210 was noted over a dose range of 80–5 mg/kg/day. Tests against the same tumour types at Bristol Laboratories confirmed these results in every respect. Therapy of mice with *L*-1210 caused a significant increase in median survival-time over controls (25 per cent or greater) at doses ranging from 80 to 3 mg/kg/day (Fig. 1). These experiments used *L*-1210 carried in the ascitic form. TCM was not effective against solid trocar implants of *L*-1210 nor was it effective against ascitic *L*-1210 if the drug was administered orally at doses up to 180 mg/kg/day. As a control procedure the possibility of carry-over of, or breakdown to, starting reactants was checked biologically. None of the starting materials or formaldehyde reaction products of *nor*HN2 or tetracycline had anti-*L*-1210 activity at doses within the stoichiometrically equivalent range of effective TCM doses (Table 1). For comparative purposes β,β′-dichlorodiethyl-*N*-methylamine (HN2) was tested against *L*-1210 and found effective at doses of

Table 1. Effect of TCM Starting Materials on Leukæmia 1210

Preparations	Dose (mg/kg/day)	Theoretically equiv. TCM dose	Response test/control Median survival time (days)	Effect (%)	Survivors (day 5)
Tetracycline HCl	176·0	200·0	8·00/7·00	114	6/6
Tetracycline HCl	88·0	100·0	7·50/7·00	107	6/6
Tetracycline HCl	58·0	78·0	7·50/7·00	107	6/6
Formaldehyde	8·0	160·0	7·00/7·00	100	6/6
Formaldehyde	2·0	40·0	7·50/7·00	107	6/6
Formaldehyde	0·5	10·0	6·50/7·00	93	6/6
Formaldehyde	0·125	2·5	7·00/7·00	100	6/6
β,β′-Dichlorodiethylamine (*nor* HN2)	80·0	338·0	11·25/7·00	161	12/12
β,β′-Dichlorodiethylamine (*nor* HN2)	40·0	164·0	8·00/7·00	114	6/6
β,β′-Dichlorodiethylamine (*nor* HN2)	20·0	82·0	7·00/7·00	100	6/6
Formaldehyde-β,β′-dichlorodiethylamine reaction product	120·0	419·0	12·00/10·00	120	6/6
Formaldehyde-β,β′-dichlorodiethylamine reaction product	80·0	280·0	10·00/10·00	100	6/6
Tetracycline–formaldehyde reaction product	130·0	164·0	8·00/7·00	114	6/6
Tetracycline–formaldehyde reaction product	57·0	73·0	7·00/7·00	100	6/6
Tetracycline–formaldehyde reaction product	26·0	32·8	7·00/7·00	100	6/6
Tetracycline–formaldehyde reaction product	11·0	13·8	6·00/7·00	86	6/6

See legend, Fig. 1. 125 per cent or greater considered 'active'.

1–0·03 mg/kg/day with a maximum increase in survival time of 47 per cent (broken line in Fig. 1). Thus HN2 was almost 100 times more potent than TCM. However, with the curves adjusted so as to be superimposed, the striking similarity of the dose-response is apparent. As a check on stability, tests against *Ca*755 were performed with TCM: (1) made up in a single solution which was then refrigerated for two weeks during the period of its use for therapy; (2) prepared fresh daily from the dry powder. The standing preparation seemed to be somewhat more potent than the fresh daily preparation regarding tumour inhibition (for example, *Ca*755 was inhibited 77 per cent at 53 mg/kg/day by the standing preparation compared to 42 per cent for a fresh daily preparation).

Since activity of TCM could not be accounted for by its starting materials the remaining possibilities are that either the intact molecule is the active agent or that TCM splits at the amide bond and releases an HN2-like compound. Though no direct chemical evidence is available at present, the following biological data and theoretical considerations tend to support the latter hypotheses. First, the standing preparation, that is, the material made up once for multiple injection, was more potent than the fresh daily preparation, suggesting instability and breakdown into a more active agent. Second, the dose-response curves of TCM and HN2 were very similar to each other. Third, others have shown that when Einhorn derivatives of tetracycline are subjected to reductive degradation under appropriate conditions, the bridging methylene group remains attached to the amino nitrogen[2,3].

Obviously the results presented here provide no evidence that TCM accumulates on neoplastic tissue as shown for other tetracyclines. However, if splitting of TCM does occur as described here, it would be of interest to determine whether the hypothesized HN2-like substance is released mostly in the injection solution, the animal's blood stream, or at the neoplastic cell.

This work was supported in part by Cancer Chemotherapy National Service Center contract *SA*-43-*ph*-2411, National Cancer Institute, U.S. National Institutes of Health.

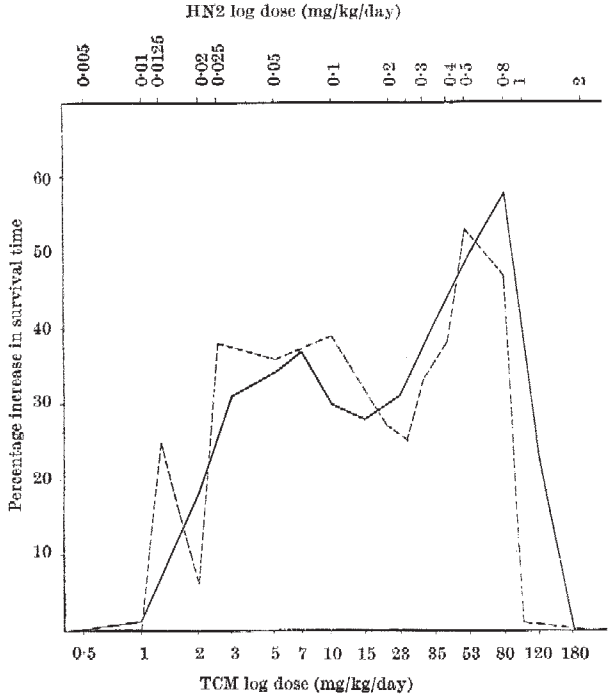


Fig. 1. *BDF*$_1$ mice inoculated day 0 with 1×10^6 ascitic *L*-1210 cells intraperitoneally. Therapy started day 1 intraperitoneally and continued once daily 13 × or until death of the animal. Survival increase of 25 per cent or greater above control ($T/C \geq 125$) considered effective for inhibition of leukæmia growth. All effective doses comprise an average of two or more experiments with at least 6 mice per treatment dose and 10 controls for each experiment. ——, TCM; – – – –, HN2

M. A. Kaplan
W. T. Bradner
F. H. Buckwalter
M. H. Pindell

Research Division,
Bristol Laboratories,
Syracuse,
New York.

[1] Rall, D. P., Loo, T. L., Lane, M., and Kelly, M. G., *J. Nat. Cancer Inst.*, **19**, 79 (1957). McLeay, J. F., *Amer. J. Surg.*, **96**, 415 (1958). Dunn, A. L., Eskelson, C. D., McLeay, J. F., Ogborn, R. E., and Walske, B. R., *Proc. Soc. Exp. Biol. and Med.*, **104**, 12 (1960). Klinger, J., and Katz, R., *Gastroenterol.*, **41**, 29 (1961). Milch, R. A., Tobie, J. E., and Robinson, R. A., *J. Histochem. and Cytochem.*, **9**, 261 (1961).

[2] Gottstein, W. J., Minor, W. F., and Cheney, L. C., *J. Amer. Chem. Soc.*, **81**, 1198 (1959).

[3] Kollar, G., Sipos, L., and Pato, G., Republic of South Africa, Patent No. *R*-61/3068, (1961).

Inhibition of 5-Hydroxytryptamine Liberation from Blood Platelets by Reserpine

Reserpine causes a release of monoamines from various tissues including isolated blood platelets[1,2]. This effect is generally attributed to an interference of the drug with the storage mechanisms for the amines. Furthermore, some authors report a protective action of reserpine against the spontaneous liberation of norepinephrine from adrenal medullary and splenic nerve granules[3–5]. The mechanism is not fully understood. It might be partly connected with the lack of glucose in the incubation medium, because this source of energy seems to be necessary for retaining the 5-hydroxytryptamine (5HT) within the platelets[6]. We have investigated the effect of reserpine on the metabolism of 5HT of isolated platelets in the absence and presence of glucose.