mouse tumours, including Ca755, and the Lewis lung tumour, can be inhibited by these compounds to varying degrees.

There was, however, little effect on ascitic tumours (even S180 in this form) when the compounds were given by routes other than intraperitoneal injection. Even by direct intraperitoneal injections neither of the compounds was able to prevent the growth of Ehrlich ascites tumour. Tests on the Walker carcinosarcoma have shown the compounds given as a single injection to be only partially effective, whereas melphalan (1 mg per kg bodyweight) as a positive control was wholly effective in producing total inhibition. Suppression of the growth of Rous sarcoma paralleled suppression of the growth of the chicks. There seemed to be no specific inhibitory activity against this tumour.

The results in Table 1 are typical of many experiments carried out with ICRF 154 and 159. They and ICRF 193 are active in oil as well as CMC and by all routes. Table 2 shows a comparison of their relative effectiveness as antitumour and immunosuppressive agents.

Attempts to produce regressions of established S180 were not successful when mice were treated for 1 week and observed for another week. There were indications, however, that a longer period of observation may be required to reveal involution of the tumour. Fig. 1 shows a clear relationship between dose and response in the case of sarcoma S180. This makes it seem likely that antitumour activity was not merely a by-product of a more general toxicity, but was the result of a specific reaction of the active compounds with some essential cell constituent or constituents.

When ICRF 154 or 159 was given to normal mice in doses greater than 30 or 100 mg/kg/day respectively there was a failure to gain and eventual loss of weight followed by death. This therefore suggests that normal cells are also vulnerable and that these substances exploit for their antitumour effects the differential rates of replication of tumour and normal cells, rather than qualitative differences between them.

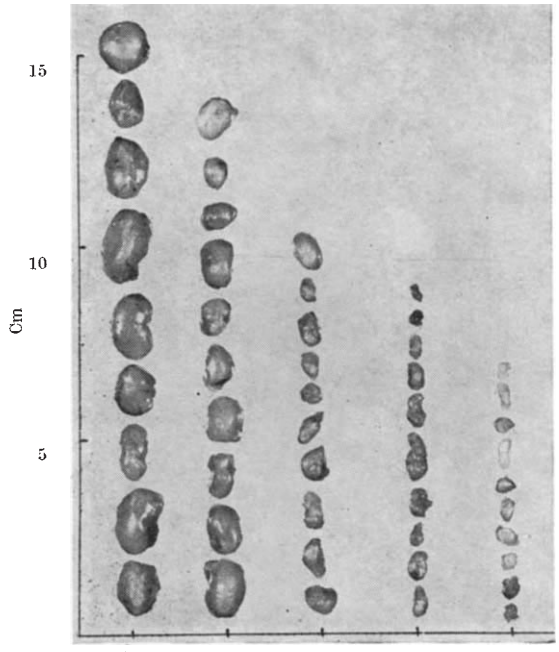
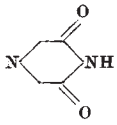


Fig. 1. Dose response relationship of ICRF 154 against S180. The tumours were dissected out and weighed 8 days after implantation. Treatment started 24 h after inoculation of tumour. Total number of doses given was five. T/C is the mean of the treated tumours divided by the mean of the weight of the control tumours.

Table 2. COMPARISON OF *bis*DIKETOPIPERAZINES AS ANTITUMOUR AND IMMUNOSUPPRESSIVE AGENTS

Code	Compound	TI	IS
(ICRF)	(given orally); $R' =$ 	(S180)	(homograft reaction)
154	$R'—CH_2—CH_2—R'$	4·4	−
159	$R'—CH_2—CH(CH_3)—R'$	9·8	±
193	$R'—CH(CH_3)—CH(CH_3)—R'$	6·7	N.T.

TI, Therapeutic index; calculated from the formula:

$$\frac{\text{Maximum tolerated dose;}}{ED_{90}}$$

ED_{90} being the dose causing 90 per cent inhibition of tumour growth). IS, Immunosuppressive activity as judged by the ability of the compounds to prolong skin allograft survival in mice. −, No immunosuppressive activity. ±, Barely significant activity. N.T., Not tested.

In this connexion it is noteworthy that neither of the compounds significantly prolonged skin homograft survival in mice (Table 2). This lack of immunosuppressive effect is of some significance because it may indicate that if the homograft reaction requires the emergence of a new generation of sensitized lymphocytes then there are some non-malignant replicating cells particularly of the lymphoid system which may after all be unaffected. These compounds are now being tested on malignancies in man.

We thank Dr R. J. C. Harris for the tests on the Rous sarcoma. One of us (A. M. C.) thanks Dr M. M. Coombs for advice and encouragement.

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1 Furst, A., in *Chemistry of Chelation in Cancer* (Thomas, Springfield, Illinois, 1963).
2 French, F. A., and Freedlander, B. L., *Cancer Res.*, **18**, 1290 (1958).
3 Petering, H. G., and Buskirk, H. H., *Fed. Proc.*, **21**, 163 (1962).
4 Mihich, E., and Nicol, C. A., *Proc. Amer. Assoc. Cancer Res.*, **4**, 44 (1963).
5 Booth, B. A., and Sartorelli, A. C., *Nature*, **210**, 104 (1966).
6 Leiter, J., Wodinsky, I., and Bourke, A. R., *Cancer Res.*, **19**, Part 2, Suppl. 368 (1959).
7 Seven, M. J., and Johnson, L. A., in *Metal-Binding in Medicine*, 154 (Lippincott, Philadelphia, 1960).
8 Frei, E., III, and Freireich, E. J., *Adv. in Chemother.*, **2**, 290 (1965).
9 Hellmann, K., Marshall, P. G., and Stayt, S., *Biochem. Pharmacol.*, **16**, 681 (1967).

Platinum Compounds: a New Class of Potent Antitumour Agents

CERTAIN platinum compounds completely but reversibly inhibit cell division in Gram-negative rods[1–4]. These compounds have been tested for antitumour activity and we report some of the preliminary results. The platinum compounds inhibit sarcoma 180 and leukaemia L1210 in mice.

The most efficacious compounds tested so far are: (1) *cis*-$Pt(IV)(NH_3)_2Cl_4$; (2) *cis*-$Pt(II)(NH_3)_2Cl_2$; (3) $Pt(II)(NH_2CH_2CH_2NH_2)Cl_2$; and (4) $Pt(IV)(NH_2CH_2CH_2NH_2)Cl_4$. These compounds were injected intraperitoneally into mice in 0·5 ml. of saline or buffer solutions. No inoculations were given to the controls, and no positive controls were used. Sarcoma 180 tests were performed in our laboratory using ICR mice and a tumour line provided by Dr S. Poilly of the NIH. Protocols of the Cancer Chemotherapy National Service Center[5] (CCNSC) were rigidly observed. The results of these tests are given in

Table 1. Because inanition could, from the recorded weight losses, cause at most a T/C × 100 value of 50 per cent[6] these compounds are effective in inhibiting the tumour. Some treated mice have been kept alive after treatment and have remained free from tumours for 6 months. Palpation indicates that the initial tumour transplant has disappeared. The mice appear normal and healthy.

Table 1. INHIBITION BY PLATINUM COMPOUNDS OF SARCOMA 180 AND LEUKAEMIA L1210

Platinum compound	Dose schedule	Mean weight change (g)	Efficacy
			Tumour mass inhibition (T/C × 100)
Sarcoma 180		Days 1–10	
I	2·5 mg/kg daily	−4·3	83
	5·0 ,, ,,	−1·6	63
	10·0 ,, ,,	−1·8	29
	Control	−2·2	[0·825 g*]
II	0·5 mg/kg daily	+0·3	75
	1·0 ,, ,,	−4·3	44
	2·0 ,, ,,	−5·6	1·8
	Control	+1·1	[0·524 g*]
III	1·25 mg/kg daily	−2·3	17
	2·5 ,, ,,	−3·6	13
	5·0 ,, ,,	−5·4	3·6
	Control	+1·1	[0·524 g*]
IV	0·62 mg/kg daily	−1·1	54
	1·25 ,, ,,	−3·8	32
	2·5 ,, ,,	−5·2	23
	5·0 ,, ,,	−5·4	20
	Control	+1·1	[0·524 g*]
Leukaemia L1210		Days 1–5	Increase in mean survival time %
I	2·5 mg/kg days 1–9	−3·3	49
II	1·25 mg/kg days 1–9	−2·7	87
	5·0 mg/kg day 1 only	−1·4	59
	10·0 mg/kg day 1 only	−3·6	> 83†

* Mean control tumour weight.

† Three of ten mice were alive and tumour free when discarded on day 30. Mean survival time of untreated controls was 9–10 days.

The activity of compounds (1) and (2) against leukaemia L1210 was evaluated by Microbiological Associates, Inc., under contract to the CCNSC in accordance with standard protocols for primary screening[5,7]. Their optimum results in the dose levels tested for BDF_1 [(C57Bl/6 × DBA/2)F_1] mice are shown in Table 1 and indicate that (2) is a potent antileukaemic agent. The National Cancer Institute is now investigating the antileukaemic activities of these compounds.

We conclude that some platinum compounds have antitumour activity. Collier and Kraus[8] found two ruthenium compounds to have "a definite effect against mouse sarcoma", and Taylor and Carmichael[9] found $RhCl_3$ inhibits mouse mammary adenocarcinoma and sarcoma. This suggests that inorganic platinum metal compounds form a new class of antitumour agents. At present, inorganic chemistry is largely unexplored for this property.

Tolerance tests which are in progress show that higher doses of these compounds affect the intestinal crypt cells, causing a gelatinous appearance of the intestines in autopsies and a reduction in the size of the spleen. These effects are reversible and animals survive if injections are stopped a few days before death would have occurred.

Tissue culture tests with Chinese hamster cells (CHEF-125) and human amniotic AV_3 cells show that incubation of these cells with the platinum compounds represses mitotic division, and distorts the chromosome morphology in the few cells that do divide. Membrane leakage also occurs.

We have, at present, no knowledge of the fate of the compounds injected into the animals or of the mechanism of action against the tumour cells.

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[1] Rosenberg, B., VanCamp, L., and Krigas, T., *Nature*, **205**, 698 (1965).
[2] Rosenberg, B., Renshaw, E., VanCamp, L., Hartwick, J., and Drobnik, J., *J. Bact.*, **93**, 716 (1967).
[3] Rosenberg, B., VanCamp, L., Grimley, E., and Thomson, A. J., *J. Biol. Chem.*, **242**, 1347 (1967).
[4] Renshaw, E., and Thomson, A. J., *J. Bact.*, **94**, 1915 (1967).
[5] *Cancer Chemotherapy Rep.*, 25 (1962).
[6] Goldin, A., Goldberg, B., Ortega, L. G., Fugman, R., Faiman, F., and Schoenbach, E. B., *Cancer*, **2**, 865 (1949).
[7] Venditti, J. M., and Abbott, B. J., *Lloydia*, **30**, 332 (1967).
[8] Collier, W. A., and Kraus, F., *Z. Krebsforsch.*, **34**, 526 (1931).
[9] Taylor, A., and Carmichael, N., *Univ. Texas Publ. No. 5314, Biochem. Inst. Studies 5, Cancer Studies 2*, 36 (1953).

Response of the Testicular Capsule to Acetylcholine and Noradrenaline

WE have used the testicular capsule as an isolated tissue preparation for pharmacological studies, and we describe here the first response of the capsule to a pharmacological agent.

The testicular capsule has long been considered an inert tissue. The very brief histological descriptions which are available imply only that the capsule is a membrane composed essentially of dense white fibrous tissue, commonly known as the tunica albuginea.

During *in vitro* metabolic studies of rat testicular tissue we have for the past 5 yr peeled off and discarded the capsule before slicing the testis with a Stadie-Riggs microtome. It recently occurred to us that it would be

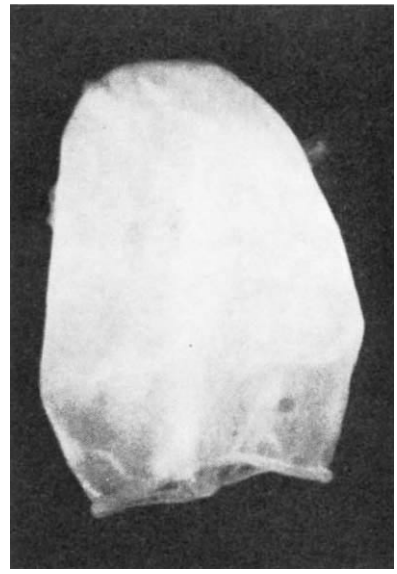

Fig. 1. Photograph of the isolated testicular capsule of the adult rat after removal of all the seminiferous tubules and underlying tissues. The capsule has been turned inside out before placing the superior and inferior threads, in order to remove completely all the interior tissue of the testis (× 3).