patients with hyperprolactinemia. Up to one-quarter of such patients harbor a pituitary adenoma (*19*). Surgery, radiotherapy, or administration of dopamine receptor agonists have been the usual forms of treatment for hyperprolactinemia. Bromocriptine is the drug most widely used; it restores normal gonadal function and fertility in nearly 80 percent of the hyperprolactinemic patients on which it is used. There is evidence that bromocriptine can also reduce pituitary tumor size (*19*).

Despite the success of bromocriptine, its use is limited; some patients fail to respond to it or develop undesirable side effects, such as gastrointestinal upset and postural hypotension. Cysteamine or similar drugs may prove beneficial in treating such patients by reducing prolactin secretion.

W. J. MILLARD
S. M. SAGAR
D. M. D. LANDIS
J. B. MARTIN
Department of Neurology, Massachusetts General Hospital, Boston 02114

T. M. BADGER
Department of Gynecology, Massachusetts General Hospital

References and Notes

1. J. R. Mitchell, S. S. Thorgeirsson, W. Z. Potter, D. J. Follow, H. Keiser, *Clin. Pharmacol. Ther.* **16**, 676 (1974); L. F. Prescott, J. Park, G. R. Sutherland, I. J. Smith, A. T. Proudfoot, *Lancet* **1976-II**, 109 (1976).
2. J. G. Thoene, R. G. Oshima, J. C. Crawhill, D. L. Olson, J. A. Schneider, *J. Clin. Invest.* **58**, 180 (1976); M. Yudkoff, J. W. Foreman, S. Segal, *N. Engl. J. Med.* **304**, 141 (1981); B. J. Corden, J. D. Schulman, J. A. Schneider, J. G. Thoene, *Dev. Pharmacol. Ther.* **3**, 25 (1981).
3. Z. M. Bacq, *Chemical Protection Against Ionizing Radiation* (Thomas, Springfield, Ill., 1965), p. 16; J. Forsberg, M. Harms-Ringdahl, L. Ehrenberg, *Int. J. Radiat. Biol.* **34**, 245 (1978).
4. H. Selye and S. Szabo, *Nature (London)* **224**, 458 (1973); S. Szabo, E. S. Reynolds, L. M. Lichtenberger, L. R. Haith, Jr., V. J. Dzau, *Res. Commun. Chem. Pathol. Pharmacol.* **16**, 311 (1977); S. Szabo, L. R. Haith, Jr., E. S. Reynolds, *Am. J. Dig. Dis. Nutr.* **24**, 471 (1979).
5. S. Szabo and S. Reichlin, *Regul. Peptides* **1** (Suppl. 1), S111 (1980); *Endocrinology* **109**, 2255 (1981).
6. S. M. Sagar, D. Landry, W. J. Millard, T. M. Badger, M. A. Arnold, J. B. Martin, *J. Neurosci.* **2**, 225 (1982).
7. J. B. Martin, P. Brazeau, G. S. Tannenbaum, J. O. Willoughby, J. Epelbaum, L. C. Terry, D. Durand, in *The Hypothalamus*. S. Reichlin, R. J. Baldessarini, J. B. Martin, Eds. (Raven, New York, 1978), p. 329; J. B. Martin, *Physiologist* **22**, 23 (1979); J. E. Morley, *Endocr. Rev.* **2**, 396 (1981).
8. Cysteamine (Sigma) doses are expressed as milligrams of the HCl salt, which was dissolved in distilled water and neutralized with 1.0*N* NaOH. The dose (300 mg/kg) was selected for the time course study because we have found (*6*) that this dose, administered subcutaneously, produces the greatest depletion of immunoreactive somatostatin in the brain without evidence of side effects, such as the development of duodenal ulcers or the induction of seizures. The animals were decapitated and the trunk blood and anterior pituitaries were harvested. Plasma was separated from the blood cells by centrifugation and stored at −30°C until being assayed for prolactin. The anterior pituitaries were sonicated for 30 seconds in 1.0 ml of phosphate-buffered saline and a portion of the supernatant was removed for the determination of prolactin. Protein levels were determined on the supernatant by the method of O. H. Lowry, J. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)].
9. Serum and anterior pituitary samples were assayed in duplicate for prolactin with materials supplied by A. F. Parlow and the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). All prolactin values are expressed in terms of the NIAMDD reference preparation Rat-PRL-RP-1 (Table 1) and Rat-PRL-RP-2 (Table 2 and in vitro data). All samples from a single experiment were assayed in the same assay to avoid interassay variations. The range of detectability in 50 μl of undiluted plasma was 1.0 to 250 ng/ml for Rat PRL-RP-1 and 0.25 to 50 ng/ml for Rat-PRL-RP-2. The significance of differences was tested with the Mann-Whitney *U* Wilcoxon nonparametric test. Significance was set at $P < .05$.
10. T. M. Badger, S. M. Sagar, W. J. Millard, J. B. Martin, P. Rosenblum, *Life Sci.* **30**, 245 (1982).
11. W. Vale and G. Grant, *Methods Enzymol.* **37**, 82 (1975).
12. Cysteamine was tested at 5, 10, 20, 60, and 120 minutes in culture. The effects of cysteamine on cell viability and prolactin were determined by incubating anterior pituitary cells with cysteamine for 1 hour, after which the cells were washed twice and incubated for an additional 24 hours in Dulbecco's minimum essential medium plus serum. At this time and following a 1-hour additional incubation period, medium and cells were harvested and frozen until the determination of prolactin levels.
13. M. G. Farquhar, in *Lysosomes in Biology and Pathology*, J. T. Dingle and H. B. Fell, Eds. (North-Holland, Amsterdam, 1969), vol. 2, p. 462; *Adv. Exp. Med. Biol.* **80**, 37 (1977).
14. D. D. Nansel, G. A. Gudelsky, M. J. Reymond, W. B. Neaves, J. C. Porter, *Endocrinology* **108**, 896 (1981); D. D. Nansel, G. A. Gudelsky, M. J. Reymond, J. C. Porter, *ibid.*, p. 903.
15. R. M. MacLeod, *Front. Neuroendocrinol.* **4**, 169 (1976); J. D. Neill, *ibid.* **6**, 129 (1980); G. A. Gudelsky, *Psychoneuroendocrinology* **6**, 3 (1981).
16. The ability of spiperone and domperidone to bind dopamine receptors was determined by identifying the dose of the receptor antagonist that could most effectively block the inhibitory effects of two dopamine receptor agonists, apomorphine and bromocriptine, on prolactin release in vitro at their (the agonists') maximal effective concentrations, and then increasing the dose of the antagonists by two and four orders of magnitude.
17. The rats were given cysteamine (300 mg/kg, subcutaneously) and anesthetized with pentobarbital (50 mg/kg, intraperitoneally) 1, 2, or 24 hours later. Then they were perfused for 15 to 20 minutes with warmed 2.0 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer (*p*H 7.4). The pituitaries were later removed, bisected, postfixed in 1.0 percent osmium tetroxide, and embedded in Epon.
18. H. B. F. Dixon, in *The Hormones*, G. Pincus, K. V. Thimann, E. B. Astwood, Eds. (Academic Press, New York, 1964), vol. 5, p. 1; U. J. Lewis, *Pharmacol. Ther.* **1**, 423 (1975).
19. M. O. Thorner and G. M. Besser, *Acta Endocrinol. (Copenhagen)* **88** (Suppl. 216), 131 (1978); R. F. Spark and G. Dickstein, *Ann. Int. Med.* **90**, 949 (1979); M. O. Thorner *et. al.*, *J. Clin. Endocrinol. Metab.* **51**, 438 (1980).
20. We thank J. Audet, C. Milbury, and L. Weinstein for excellent technical assistance. Spiperone and domperidone were gifts from Janssen Pharmaceutica, Beerse, Belgium. Supported by PHS grant AM 26252.

23 February 1982

Cure of Mice Infected with *Trypanosoma rhodesiense* by *cis*-Diamminedichloroplatinum (II) and Disulfiram Rescue

Abstract. *Mice infected with* Trypanosoma rhodesiense *were treated concurrently with* cis-*diamminedichloroplatinum (II) (DDP), disulfiram, and hydration. Most of the mice (92.5 percent) were cured; inoculation of blood or suspensions of brain or heart from these animals did not produce disease in recipient mice. The dose of DDP needed to eliminate the trypanosomes, 3 milligrams per kilogram of body weight per day for 7 days, was lethally toxic unless the animals received disulfiram orally and subcutaneous injections of physiologic saline, which reduced the acute renal necrosis caused by DDP alone. Some mild to moderate reversible renal damage was noted upon pathologic examination of the treated mice.*

Several antineoplastic compounds show antitrypanosomal activity in vivo (*1, 2*). One of these agents is *cis*-diamminedichloroplatinum (II) (DDP). Experiments with mice indicated that, although infected animals were not completely cured by DDP, the drug did prolong their life (*1*). This suggested that the DDP had trypanocidal activity but that the doses administered were inadequate to eradicate all of the infecting parasites.

Methods have recently been devised to ameliorate or ablate the adverse effects of DDP, primarily renal tubule toxicity, previously observed at therapeutic doses (*3*). These methods include hydration and administration of compounds thought to preferentially block DDP's toxic effects without blunting its antineoplastic action (*4*). Diethyldithiocarbamate, a metabolite of disulfiram (Antabuse), is perhaps the best characterized of these substances. Its protective action is thought to derive from the chelation of DDP prior to the generation of irreversible nephrotoxicity. These protective agents allow greater doses of DDP to be administered without concomitant increases in nephrotoxicity, thus enhancing DDP's therapeutic index. They also make it possible to examine the curative potential of DDP in trypanosome-infected animals treated with substantially increased doses.

In studies of the effects of DDP on mice (ICR/Ha Swiss) infected with *Trypanosoma rhodesiense*, we first measured the activity of the drug against the infecting organism in vitro. Parasites were collected from male Walter Reed rats by cardiac puncture and isolated by using a dimethylaminoethyl cellulose anion exchange column. Elution was effected with 0.1*M* phosphate-buffered sa-

0036-8075/82/0730-0454$01.00/0

line, and the organisms were washed and resuspended in RPMI-1680 medium containing 33 percent heat-inactivated horse serum (2.0×10^8 trypanosomes per milliliter).

The ability of DDP to inhibit the uptake of [*methyl*-^{3}H]thymidine (49.5 mCi/mmole) or L-[U-^{14}C]leucine (355 mCi/mmole) was quantified by the method of Desjardins (*5*). Trypanosomes (5×10^6) were incubated in medium containing either 2.5 μCi of [^{3}H]thymidine or 0.5 μCi of [^{14}C]leucine per milliliter with or without DDP (12 μg/ml) or the antitrypanosomal agent ethidium bromide (0.65 to 0.98 μg/ml). DDP had no effect on the uptake of the radioactive precursors over a 3-hour incubation period. Ethidium bromide inhibited both [^{3}H]thymidine and [^{14}C]leucine incorporation into trypanosomes. Despite its lack of effect on radioactive precursor uptake, DDP rendered the exposed trypanosomes noninfective to recipient mice. This indicated that DDP had some delayed effect on the parasite's DNA or protein synthesis or had some other effect on the parasite or host. It did confirm the antitrypanosomal potential of DDP.

Table 1 shows the effects of DDP on mice harboring fatal trypanosomal infections and on parasite-free animals. When the amount of DDP administered was less than 3 mg per kilogram of body weight per day the animal's blood was not cleared of infecting organisms (data not shown). Five to six consecutive daily treatments with 3 mg/kg-day did not reliably cure the mice of trypanosomiasis as indicated by the occurrence of late relapses of the disease. Seven daily doses of DDP did cure the mice of trypanosomiasis. However, some of the uninfected animals that received six or more daily doses of DDP without hydration and disulfiram died. Only the combination of seven consecutive daily doses of DDP with hydration and disulfiram reliably cured the mice of trypanosomiasis and produced acceptable levels of toxicity.

Table 1. Effect of DDP on mice infected with *T. rhodesiense* (1×10^6) and on uninfected control mice. Thirty minutes after the mice were infected with trypanosomes (1×10^6), therapy with DDP was begun. Each mouse received an intraperitoneal injection of 3 mg of DDP per kilogram of body weight per day. Disulfiram (250 mg/kg in a saline and glycerol mixture, 1:4 by volume) was given orally exactly 4 hours after each DDP dose. Hydration was achieved by giving the mice a subcutaneous injection of physiologic saline (3 ml) four times per day. Survival is expressed as the percentage of mice surviving 30 days after the initial dose of DDP. All groups included ten mice except where indicated.

Duration of therapy (days)	Uninfected mice			Infected mice		
	No hydration (%)	With hydration (%)	Hydration plus disulfiram (%)	No hydration (%)	With hydration (%)	Hydration plus disulfiram (%)
5	100			10		
6	10	100	100	0	0*†	62.5*†‡
7	0	90	100‡	0*	60*	92.5‡

*Blood of all mice was cleared of trypanosomes. †Relapse of parasitemia. ‡Average survival from four experiments with ten mice per experiment.

The possibility that trypanosomes remained latent in the blood, heart, or brain of the apparently cured animals (surviving at 30 days) was examined by inoculating suspensions of these tissues into uninfected recipient animals. None of the recipient animals developed trypanosomiasis.

Although DDP with hydration and disulfiram appeared to eradicate trypanosomes from these mice, the toxic effects of the drug were not completely eliminated. A study of the effects of disulfiram rescue and hydration on DDP toxicity (*6*) indicated that, at 30 days after the initial treatment with DDP, kidney damage was at a minimum when disulfiram was combined with hydration. Disulfiram alone was superior to saline alone in ameliorating DDP toxicity as measured by survival at 30 days and according to a histopathological evaluation. Disulfiram plus saline produced plasma BUN levels within the normal range over the entire 30-day period when the daily dose of DDP was 3 mg/kg, whereas the control mice that received no DDP produced markedly elevated BUN levels.

Acute tubular necrosis was demonstrable after 8 days of treatment with DDP alone (3 mg/kg-day) (Fig. 1A). Minimal tubulointerstitial nephritis occurred when the animals received disulfiram and saline in addition to DDP (Fig. 1B). Histological examination of gastrointestinal tissue sections revealed no toxic effects of the drugs.

The toxicity of DDP was further re-

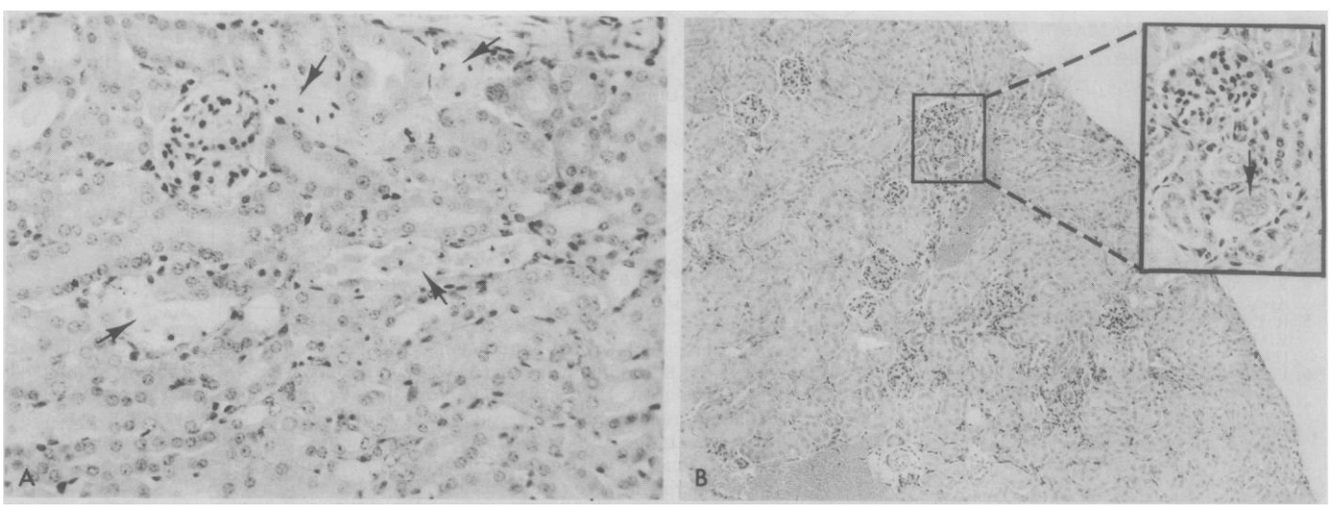

Fig. 1. (A) Photomicrograph of a section of kidney from a mouse infected with *T. rhodesiense* 8 days previously and treated with DDP. The arrows point to areas of acute tubular necrosis, showing epithelial cells with pycnotic nuclei and desquamation into the tubular lumen (×550). (B) Photomicrograph of section of kidney of mouse infected with *T. rhodesiense* 32 days previously and treated with DDP, disulfiram, and saline. Minimal tubulointerstitial nephritis is shown by focal accumulations of mononuclear inflammatory cells surrounding areas of regenerating tubular epithelium as indicated by arrow (×100) (inset, ×250).

duced by embedding the drug in a polylactic acid matrix (PLA) (*7*). A single intraperitoneal injection of DDP-PLA (100 mg/kg, in saline) cured the mice of *T. rhodesiense* (*8*). The amount of DDP released from the DDP-PLA matrix composite during the first week after the injection was less than the amount of drug administered for a single day during the 7-day regimen of 3 mg/kg-day. Consequently, the drug dose released from the composite was below the nephrotoxic levels for DDP.

Thus we have shown that DDP administered in conjunction with disulfiram rescue and hydration is an effective antitrypanosomal agent. Although the treatment was toxic to 3 of 40 animals (7.5 percent, Table 1), most of the animals were cured and their tissues did not harbor latent infectious organisms.

Trypanocidal effects of antibiotic and antineoplastic agents have been noted in several recent reports (*9*). We now add DDP, an antineoplastic agent commonly used in humans, to the list of trypanocidal agents with potential for application in vivo. The specific regimen that we used to ameliorate potential toxicity may prove useful in the treatment not only of infectious diseases but also of human malignancies.

MICHAEL S. WYSOR
Department of Parasitology, Walter Reed Army Institute of Research, Washington, D.C. 20012
LEONARD A. ZWELLING
Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
JAMES E. SANDERS
Department of Pathology, Walter Reed Army Institute of Research
MARIE M. GRENAN
Department of Parasitology, Walter Reed Army Institute of Research

References and Notes

1. K. E. Kinnamon and E. A. Steck, *Antimicrob. Agents Chemother.* **15**, 157 (1979).
2. ———, D. S. Rane, *J. Natl. Cancer Inst.* **64**, 391 (1980).
3. J. A. Gottlieb and B. Drewinko, *Cancer Chemother. Rep.* **59**, 621 (1975).
4. R. F. Borch and M. E. Pleasants, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6611 (1979).
5. R. E. Desjardins, R. A. Casero, G. P. Willet, G. E. Childs, C. J. Canfield, *Exp. Parasitol.* **50**, 260 (1980).
6. For details, see M. S. Wysor, J. E. Sanders, L. A. Zwelling, R. F. Borch, in preparation.
7. S. Yolles and M. F. Sartori, in *Drug Delivery Systems*, R. L. Juliano, Ed. (Oxford Univ. Press, Oxford, 1980), p. 84.
8. M. S. Wysor *et al.*, in preparation.
9. D. E. Strickler and C. L. Patton, *Proc. Natl. Acad. Sci. U.S.A.* 77, 1529 (1980); J. Williamson, T. J. Scott-Finnigan, M. A. Hardman, J. R. Brown, *Nature (London)* **292**, 466 (1981); S. G. Baum, M. Wittner, J. P. Hadler, S. B. Horowitz, J. D. Dennis, P. B. Schiff, H. B. Tanowitz, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4571 (1981).

9 November 1981; revised 10 May 1982

Small Nuclear RNA U2 Is Base-Paired to Heterogeneous Nuclear RNA

Abstract. *Eukaryotic cells contain a set of low molecular weight nuclear RNA's. One of the more abundant of these is termed U2 RNA. The possibility that U2 RNA is hydrogen-bonded to complementary sequences in other nuclear RNA's was investigated. Cultured human (HeLa) cells were treated with a psoralen derivative that cross-links RNA chains that are base-paired with one another. High molecular weight heterogeneous nuclear RNA was isolated under denaturing conditions, and the psoralen cross-links were reversed. Electrophoresis of the released RNA and hybridization with a human cloned U2 DNA probe revealed that U2 is hydrogen-bonded to complementary sequences in heterogeneous nuclear RNA in vivo. In contrast, U2 RNA is not base-paired with nucleolar RNA, which contains the precursors of ribosomal RNA. The results suggest that U2 RNA participates in messenger RNA processing in the nucleus.*

The small nuclear RNA's are abundant, metabolically stable low molecular weight RNA species that are present in all eukaryotes (*1*). Their functions are not understood. The first small nuclear RNA to be purified was the species termed U2 ("U" for uridylate-rich), which is 189 nucleotides long, and like most of the other small nuclear RNA's, carries an inverted trimethylguanosine "cap" at its 5′ end (*2*). We have investigated the possibility that U2 RNA is associated with high molecular weight nuclear RNA [heterogeneous nuclear RNA (hnRNA)] through intermolecular base-pairing of complementary se-

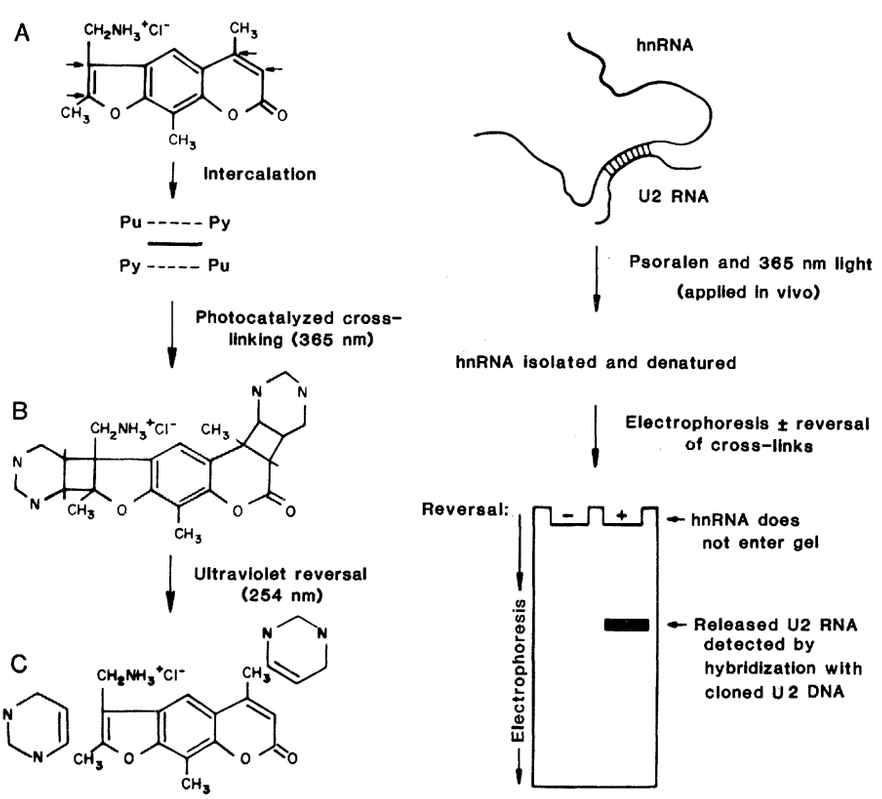

Fig. 1. Psoralen cross-linking and experimental strategy. (Left) Chemistry of cross-linking. 4′-Aminomethyl-4,5′,8-trimethylpsoralen (A) was synthesized from 4,5′,8-trimethylpsoralen as described (*3*, *5*). The arrows indicate carbon atoms in the pyrone and furan rings which, upon 365-nm irradiation, react with the carbons at positions 5 and 6 of pyrimidines to form cyclobutane bridges. In the case of diadducts, this constitutes a covalent, interstrand cross-link (B). The cross-links can be subsequently broken by irradiation at 254 nm (*7a*, *8*), as shown in (C). (Right) Base-pairing between small nuclear RNA U2 and high molecular weight hnRNA is detected by treating intact cells with 4′-aminomethyl-4,5′,8-trimethylpsoralen and 365-nm light. The hnRNA is then extracted and denatured, and equal amounts are subjected to electrophoresis with or without reversal of cross-links. The polyacrylamide concentration of the resolving gel is such that high molecular-weight RNA is excluded. Small RNA species released by reversal of psoralen cross-links are identified after electrophoresis by hybridization with cloned DNA specific for individual small nuclear RNA species.

0036-8075/82/0730-0456$01.00/0