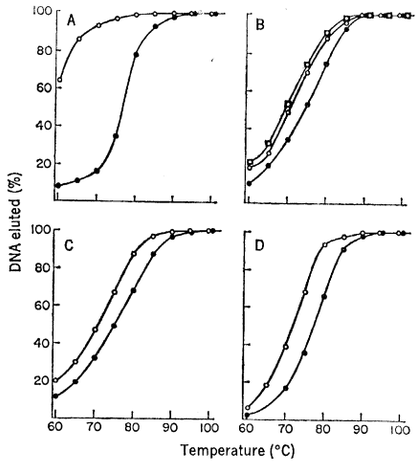
Fig. 1. Thermal stability of homologous and heterologous viral RNA · DNA hybrids. Reassociation reactions were performed as described in legend to Table 2 with DNA (3000 to 5000 count/min) and 0.6 μg of viral RNA. The mixtures were then diluted to a PB concentration of 0.12*M* and passed through a HAP column at 60°C. Thermal elution profiles were obtained by raising the column temperature in 5°C increments and washing with three 2-ml portions of 0.12*M* PB at each temperature. Radioactivity in each fraction was determined as before. (A) DNA prepared from Rauscher murine leukemia virus RNA was hybridized with Rauscher virus RNA (closed circles) and Rickard feline virus RNA (open circles). (B) DNA prepared from Moloney murine leukemia virus RNA was hybridized with Moloney virus RNA (closed circles), Rauscher virus RNA (open circles), and AKR murine leukemia virus RNA (squares). (C) DNA prepared from Rauscher virus RNA was hybridized with Rauscher virus RNA (closed circles) and Moloney virus RNA (open circles). (D) DNA prepared from AKR murine virus RNA was hybridized with AKR virus RNA (closed circles) and Moloney virus RNA (open circles).

identify each virus type and distinguish it from other closely related viruses. This relatively simple and rapid procedure should enable more sophisticated molecular experimentation on the nature of "endogenous" mammalian viruses (*14*) and putative molecular recombination between virus strains as an adjunct to genetic experiments. Other hybridization experiments in our laboratory have shown that murine virus DNA prepared from viruses grown in cat cells (*13*) hybridizes efficiently with RNA from murine viruses grown in mouse cells and has an equal thermal stability to that of the homologous hybrid (*15*). These experiments show that the nucleic acids characterized in these hybridization experiments are virus-specific, since the same RNA is incorporated into virus particles (and copied by the reverse transcriptase enzyme) regardless of the cell species in which the virus is grown.

We report here that the human "candidate" virus RD-114 is genetically distinct from the strains of feline and murine viruses studied. The RD-114 virus was obtained after the inoculation of human rhabdomyosarcoma cells into the brain of a kitten in utero (*2*). After these human cells were recovered from the resulting tumors, they were producing high titers of nontransforming C-type virus particles. The fact that these particles are not closely related to the two feline viruses examined in-



dicates that they are either (i) a new feline virus unrelated to the Rickard and Gardner strains or (ii) a virus of human origin. We feel the first possibility is correct because we have been able to chemically induce a C-type virus from a cat cell line which is indistinguishable from RD-114 virus (*16*).

The studies reported here form the basis for a taxonomic scheme based on the molecular relatedness between various mammalian RNA tumor viruses. These studies can be expanded by including other laboratory strains of viruses whereupon any new isolate, regardless of the species of cell in which it is grown, can be identified by comparing its genome to those of other viruses. This technique should aid the identification of viruses from human neoplasias.

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Drug-Induced Change in the Distribution of Sulfonamides in the Mother Rat and Its Fetus

Abstract. *The distribution of a highly bound antibacterial sulfonamide was markedly altered in both the mother rat and its fetus by interfering with the binding of this drug to plasma protein in the mother. This effect was due to binding displacement, since the displacing agent had little or no effect on the distribution of another sulfonamide with very low binding to plasma protein.*

Binding to plasma protein not only can interfere with the biological activity of drugs (and endogenous substances), but also it is one of the major factors that influences the distribution of a drug to other body compartments (*1*). It has been shown that the antibacterial activity in vitro and the tissue levels of highly bound sulfonamides in the rat could be significantly increased by intentionally displacing them from binding to protein with another highly bound drug (*2*). Since then, several case reports have

appeared of unexpected toxic reactions to drugs because of their inadvertent displacement from binding to protein, resulting in the sudden increase in the unbound active fraction after the administration of another highly bound drug (*3*). The actual frequency of occurrence of such clinical mishaps is unknown, but possibly related to this type of drug interaction is the observation that the incidence of toxic effects increases dramatically with the number of drugs patients take simultaneously (*4*). In a related study we showed that several drugs together can, by an additive effect, displace another drug from binding to protein under conditions in which each shows little displacing activity (*5*).

In order to evaluate the possibility that one drug might modify the pharmacology of another drug in utero, we determined whether a displacing agent could alter the distribution of a highly bound antibacterial sulfonamide between a mother and its fetus. We did the experiments in pregnant Sprague-Dawley rats (350 to 400 g) on day 20 of gestation. For comparative purposes we used sulfamethoxypyridazine (SMP), a highly bound sulfonamide (more than 75 percent bound at the concentrations we were using), and sulfanilamide (SNM), one of the least bound drugs (less than 20 percent) in this class (*2*). As a displacing agent, we used sulfinpyrazone (SPZ), which we had previously found to be one of the most active drugs for this purpose (*2*). Following the earlier protocol (*2*), an initial large dose (25 mg per kilogram of body weight) of the sulfonamide was administered by intracardiac injection and then we gave 5 to 10 mg/kg subcutaneously every 15 to 30 minutes to maintain a constant plasma concentration of sulfonamide for 75 minutes in order to approach drug equilibrium between mother and fetus. The rats were lightly etherized each time an injection was given or a blood sample taken. Using a heparinized syringe, we sampled blood from the heart at 30 and 75 minutes after the initial injection of sulfonamide. After the 75-minute sample, we gave SPZ (50 mg/kg) by intracardiac injection, and 30 minutes thereafter we obtained the final blood sample and then decapitated the animal. We quickly removed the fetuses and dissected each into head and body sections. Grossly incomplete or partially reabsorbed fetuses were not used. We removed the major organs from the mother rat and centrifuged the blood for plasma. We performed the dissections on ice and froze all specimens until analyzed (within 24 hours) for sulfonamide content (diazotizable amine) by a modification of the Bratton and Marshall method (*2*). Although the dose of SPZ in these experiments, which was the same as that used previously (*2*), is about five times that used clinically, it is not unreasonable in terms of the relative drug dosage applied to the rodent model.

From the results tabulated in Table 1, A and B, we can make these conclusions: (i) SNM is well distributed between mother and fetus, apparently throughout body water; (ii) the distribution of SNM is not significantly altered by the displacing agent [in an earlier study (*2*) it was shown that SPZ did not change the plasma concentrations of SNM in the male rat]; (iii) SMP is poorly distributed in the mother and fetus and apparently is restricted to the extracellular compartment; (iv) the distribution pattern of this sulfonamide is markedly altered by the displacing agent so that the ratio of tissue to plasma approaches unity and it now more closely resembles SNM [these data are in good agreement with those previously obtained in the male rat (*2*)]; and (v) the effect of SPZ on SMP is due to displacement from plasma protein binding sites.

The effect of SPZ on SMP is much more striking on the basis of the ratios of tissue to plasma because of the marked drop in plasma concentration of the sulfonamide. Because of the large volume into which the unbound sulfonamide is distributed after displacement from binding, the absolute increase in tissue concentrations is less impressive. In Table 1B note the difference in the concentration of SMP between the mother's brain and the head of the fetus, both in the absence and presence of SPZ. (The level of significance of these differences is not shown but in both cases $P < .001$.) Since the plasma concentrations of sulfonamide, at least prior to SPZ, would be expected to be similar in the mother and the fetus (*6*), this difference in tissue concentrations is probably due to the presence of a mature, more restrictive blood-brain barrier in the mother (*7*). An alternative explanation is that if the binding of SMP is less in fetal plasma than in the mother's plasma, more of the unbound fraction would be available for diffusion into the head (brain) (*8*) of the fetus. In

Table 1. Distribution of sulfonamides in mother and fetus after displacement from binding to protein. Each value ± 1 standard error is the mean from four rats. The displacing agent, sulfinpyrazone (SPZ), was given by intracardiac injection 75 minutes after the initial sulfonamide administration and the rats were killed 30 minutes after the SPZ; NS, not significant.

	Before SPZ		After SPZ		Significance*	
	Concentration (mg/100 ml)	Ratio of tissue to plasma	Concentration (mg/100 ml)	Ratio of tissue to plasma	Concentration (*P* value)	Ratio (*P* value)
			(*A*) *Sulfanilamide*			
Mother						
Plasma	2.8 ± 0.07		2.3 ± 0.09		NS	
Brain	2.0 ± .08	0.73 ± 0.05	1.8 ± .13	0.82 ± 0.07	NS	NS
Muscle	2.4 ± .19	.87 ± .09	2.1 ± .09	.95 ± .05	NS	NS
Spleen	2.4 ± .14	.88 ± .07	2.3 ± .08	1.00 ± .04	NS	NS
Kidney	3.9 ± .17	1.40 ± .08	3.6 ± .21	1.60 ± .09	NS	NS
Uterus	1.8 ± .14	.65 ± .04	1.8 ± .20	0.82 ± .10	NS	NS
Fetus						
Head	2.1 ± 0.21	0.75 ± 0.07	1.9 ± 0.14	0.83 ± 0.05	NS	NS
Body	2.2 ± .11	.79 ± .02	2.0 ± .04	.90 ± .02	NS	< .01
Placenta	2.1 ± .11	.77 ± .03	2.0 ± .10	.89 ± .03	NS	< .05
			(*B*) *Sulfamethoxypyridazine*			
Mother						
Plasma	10.3 ± 0.75		4.8 ± 0.24		< .001	
Brain	1.3 ± .10	0.14 ± 0.02	1.7 ± .04	0.37 ± 0.03	< .02	< .001
Muscle	2.1 ± .06	.20 ± .02	3.0 ± .08	.62 ± .05	< .001	< .001
Spleen	3.6 ± .09	.35 ± .03	4.0 ± .08	.86 ± .06	< .01	< .001
Kidney	4.3 ± .33	.43 ± .06	4.1 ± .16	.87 ± .08	NS	< .01
Uterus	3.6 ± .23	.36 ± .05	3.7 ± .26	.77 ± .02	NS	< .001
Fetus						
Head	2.3 ± 0.19	0.23 ± 0.02	3.8 ± 0.20	0.80 ± 0.06	< .01	< .001
Body	2.9 ± .13	.28 ± .03	3.6 ± .44	.76 ± .11	NS	< .01
Placenta	3.8 ± .36	.37 ± .03	3.7 ± .33	.79 ± .10	NS	< .01

*The level of significance between the means before and after the administration of SPZ was calculated by Student's *t*-test (two-tailed). The *P* values were calculated for both the absolute concentrations of sulfonamide as well as for the ratios of tissue to plasma relative to the mother's plasma.

man, at least, the plasma protein bindings of drugs in the fetus and neonate has been reported to be less than in the adult (*9*). Such data are not available for the rat but the plasma concentration of albumin [the main drug-binding component of plasma (*1, 2*)] is less in the fetal rat than in the adult (*10*), and there is a relation, although not a proportional one, between binding and albumin concentration (*1, 5*).

An indication of the potential hazard to the fetus and newborn from this type of interaction comes from the report in 1956 of the production of fatal kernicterus in several premature infants who were given sulfisoxazole as part of a prophylactic antibacterial regimen (*11*). This highly bound sulfonamide displaced sufficient bilirubin from binding to plasma protein to cause a toxic concentration to be reached in the brain of susceptible infants. Although this did not happen in utero, the data in Table 1, A and B, attest to the possibility of such an occurrence, particularly in light of the deficient binding in the fetus and neonate previously noted (see above). In addition, we had reported earlier that displacing agents are more effective in the presence of a deficient binding (*5*). It is also conceivable that the displacement of potentially hazardous, highly bound drugs in the mother may play some role in the production of birth defects and in the relatively high fetal mortality rate in this country. Recently, Bleyer *et al.* (*12*) published a list of the drugs commonly ingested by mothers during pregnancy. Some of these drugs are highly bound to plasma protein and a number have been shown experimentally to have a deleterious effect on the fetus (*13*). In light of our limited knowledge and experience with drug interactions in man, the decision to administer drugs to a pregnant patient for treatment of a minor complaint should be weighed against the potential hazard to the fetus.

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Nematode Morphogenesis: Localization of Controlling Regions by Laser Microbeam Surgery

Abstract. *Laser microbeam studies reveal that postembryonic development of the free-living nematode* Panagrellus silusiae *is under the control of specific regions. Growth is regulated by the hindgut, and ecdysis by nerve cell bodies situated anterior to the nerve ring, and gonad development is under the control of the nerve ring. This latter event is presumably neuronally mediated, while the other events are under hormonal control.*

In the free-living nematode *Panagrellus silusiae* the major events of postembryonic development are growth, cuticle formation, ecdysis, gonad development, and the development of sexual behavior (*1*). Coordinate control of these events has been postulated. Such control could be either hormonal or nervous. However, as nematodes are eutelic, with little or no capacity for regeneration, with a high internal hydrostatic pressure, few experimental studies have been carried out to determine the possible sources of coordination of postembryonic development. Ligature and microirradiation studies of larger animal parasitic species have demonstrated that the general region of the nerve ring controls the events of exsheathment (*2*). Laser microbeam irradiation has been used to determine the receptor site for mating attraction (*3*). This technique is most suited to the nematode system, as a high-energy flux is delivered to the tissue in a localized region without disruption of the body wall and the resultant loss of internal pressure.

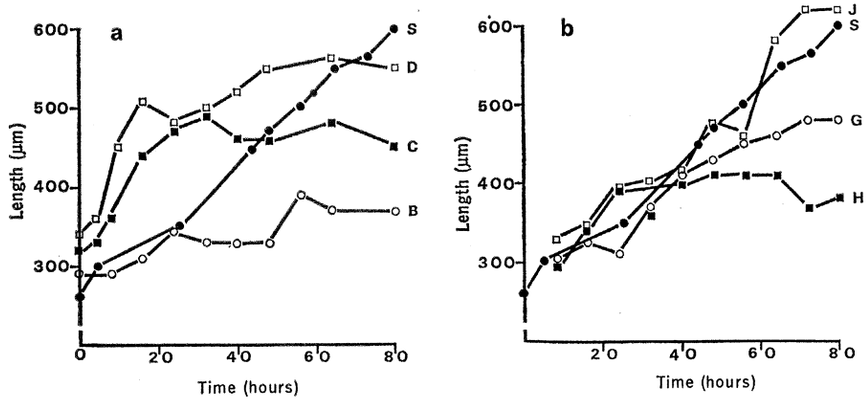

Fig. 1. Growth of individual nematodes after laser microbeam irradiation with a 7-μm spot (260-joule input). (a) Growth after irradiation in anterior region; (b) effects of irradiation in posterior region. Key to the regions indicated: *B*, cell bodies of the nerve ring; *C*, nerve ring; *D*, pharynx; *G*, gonad primordium; *H*, hindgut; *J*, extreme posterior region of the nematode; *S*, sham (laser spot delivered to medium near nematode).