thought it of interest to compare the chromosome banding patterns of early mouse embryos with those of differentiated mouse cells.

Female mice (*Mus musculus*) (CF-1; Carworth, Inc.) were superovulated and placed with CF-1 males when human chorionic gonadotrophin (HCG) was given. Mated females were injected with 50 μg of colchicine; the interval between HCG and colchicine administration was 28 hours for first cleavage embryos, 52 hours for second cleavage embryos, 72 hours for eight-cell embryos, and 100 hours for blastocysts. Four to five hours after injection of colchicine, embryos were flushed from the fallopian tubes or uteri, exposed to 1 percent sodium citrate, and fixed in methanol : acetic acid (3 : 1) (*9*). Slides were than stained with QM (100 μg/ml), and suitable chromosome spreads were located and photographed with the fluorescence microscope (*1*).

Figure 1 shows a karyotype prepared from an embryo at late prophase of the first cleavage division, when the maternally and paternally derived chromosome groups are still separate (*10*). The less condensed paternal chromosomes exhibit the same banding patterns as do the more contracted maternal ones. We also observed five other complete and several incomplete cells at first cleavage, about ten incomplete cells from second cleavage embryos, five complete cells from eight-cell embryos, and eleven complete cells from blastocysts. In all these embryonic stages the banding patterns are the same as those found in mouse fibroblasts and bone marrow cells (*1, 2*).

It has been suggested (*11*) that regions of chromosomes which fluoresce relatively brightly after QM staining are in general the same as those regions which replicate relatively late. Since late replication is a property generally attributed to heterochromatin, this suggests in turn that the brightly fluorescent regions contain heterochromatin. However, the fluorescent pattern of the facultative heterochromatin of the inactive X chromosome in mammalian females is the same as that exhibited by the euchromatic, active X (*3*). Insofar as the bright-staining regions can be taken to represent constitutive heterochromatin (*11*), our results indicate that its chromosomal distribution is established by the time chromosomes are first seen in the embryo, about 16 hours after fertilization. Our results therefore add data on the earliest embryonic stages to the accumulating evidence that the feature of chromosome structure which is revealed by QM staining does not change during development or differentiation.

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Vampire Bat Control by Systemic Treatment of Livestock with an Anticoagulant

Abstract. *The blood of beef cattle given single doses (1 milligram per kilogram of body weight) of diphenadione (2-diphenylacetyl-1,3-indandione) became toxic to vampire bats (*Desmodus rotundus*) and remained toxic for 3 days without harming the cattle. Cattle at three ranches in Mexico treated with single intraruminal injections of diphenadione experienced a reduction in vampire bat bites of 93 percent. Bioassays of milk and liver from cattle treated orally with diphenadione in the laboratory indicated that there were no residue problems.*

We have developed a method of reducing populations of vampire bats that attack cattle by treating cattle systemically with a single intraruminal dose (1 mg/kg) of an anticoagulant, diphenadione (2-diphenylacetyl-1,3-indandione) (*1*). Vampire bats (Desmodontidae) feed exclusively on the blood of live vertebrates. The family's three genera, particularly *Desmodus*, the most abundant, are important carriers of paralytic rabies, the most serious animal health problem in Latin America; losses attributable to rabies, estimated at a million head of cattle a year, plus malnutrition, myiasis, and loss of blood resulting from vampire bat attacks, cost the Latin American cattle industry up to $250 million annually (*2*).

Mass vaccination of cattle has been the principal weapon for rabies control (*2*). Before 1970, no specific method for reducing vampire bat populations was known (*3*), even though control efforts with gassing, poisoning, shooting, netting, trapping, and dynamiting date back to 1934 (*2*). In 1968, the U.S. Bureau of Sport Fisheries and Wildlife, under an agreement with the U.S. Agency for International Development and the government of Mexico, initiated research to develop effective, economical, and safe methods for reducing the attacks of vampire bats on livestock through population reduction. One technique developed in this program has been described by Linhart *et al.* (*4*). Bats are mist-netted near cattle, treated topically with petroleum jelly containing a slow-acting toxicant, and released; when treated bats return to the roost, the toxic jelly is spread to other members of the colony and is ingested in grooming. The second method, described here, capitalizes on the daily requirements of vampire bats for blood, the tendency of anticoagulants to temporarily bind to the blood protein, and the differential sensitivity of cattle and vampire bats to diphenadione. In laboratory studies with caged (unexercised) common vampire bats (*Desmodus rotundus*) given diphenadione, we found that the single oral LD_{50} (dose statistically estimated as lethal to 50 percent of the population) is 0.91 mg/kg, but that a single oral dose of up to 5 mg/kg in cattle produces no observable signs of intoxication beyond a moderate increase in the clotting time of plasma prothrombin (the clotting time of whole blood was essentially unaffected). In one experiment, as little as 1 mg/kg of diphenadione was injected into the rumen of three beef calves (6 to 9 months old), and blood samples were taken at 24-hour intervals after treat-

ment; single 20-g feedings of the defibrinated blood killed three out of three common vampire bats—one at 24, one at 48, and one at 72 hours—and one of three bats at 96 hours.

The effectiveness of the technique in reducing vampire bat attacks on cattle was demonstrated at three adjoining ranches encompassing about 3.9 km^2 in the state of San Luis Potosí, Mexico. The cattle, mixed-breed range stock, were under moderately severe attack by common vampire bats; fresh bites (those obviously less than 24 hours old) averaged 1.2 per animal 30 days before treatment and 1.1 per animal at the time of treatment. According to the ranch owners, the herds had a long history of vampire bat bites with no observed fluctuation in biting intensity. On 10 and 11 February 1972, 207 cows, calves, and bulls (essentially all of the ranches' stock) were either tethered or herded into chutes. The number of fresh bites on each animal was recorded, the body weight was estimated, and 1 mg/kg of diphenadione was injected into the rumen compartment of the stomach with a pistol-grip automatic syringe fitted with a 14-gauge, 1.5-inch (3.8-cm) disposable needle. For injection, diphenadione was added to a neutralized suspension of 0.05 percent Carbopol 941 (*5*) in water at a concentration of 50 mg/ml. Cattle were released to pasture after treatment. About 2 weeks later, the cattle were again examined for fresh bites and general condition.

The results of these trials are summarized in Table 1. Two weeks after treatment, fresh bites were reduced 93 percent, to a mean of 0.07 bite per animal. Chi-square analysis showed that this reduction was highly significant ($P < .01$) and that the treatment was equally effective at the three ranches. The cattle exhibited no ill effects from the treatment 2 weeks after treatment, and the ranchers reported none earlier. Previous studies at 11 areas in Mexico showed that the number of fresh bites on cattle is highly correlated ($r = .92$) with the number of vampire bats captured in mist nets around the cattle. According to the prediction equation derived in these studies, 214 bites indicate a feeding population of 212 vampire bats, and 15 bites, 15 bats. Each wild vampire bat may consume an average of 20 ml of blood per day (*6*). On this basis, we estimate that the average amount of blood lost from one treated animal was reduced from 20.5 ml a day to 1.4 ml.

Table 1. Reduction in vampire bat attacks when cattle at three Mexican ranches were given single intraruminal injections of diphenadione (1 mg/kg).

Ranch	Immediately before treatment		After treatment			
	Animals examined (No.)	Fresh bites (No.)	Days after treatment	Cattle examined (No.)	Fresh bites (No.)	Reduction in bites (%)
1	93	80	13	84	5	93.8
2	71	88	14	77	9	89.8
3	43	46	12	49	1	97.8
			Totals and mean			
	207	214		210	15	93.0

Initial laboratory tests suggest that there are no long-term residue problems associated with the use of diphenadione in beef cattle. The liver is the main site of action of indirect anticoagulants such as diphenadione. Therefore, the livers of two beef calves slaughtered 30 days after they had been given a 2.75-mg/kg oral dose of diphenadione were fed to laboratory rats, which are extremely sensitive to diphenadione [long-term oral dose causing 50 percent mortality, 0.2 mg/kg per day (*7*)]. Thirty rats were fed this liver for seven consecutive days. Consumption averaged 693.4 g per kilogram of body weight for ten rats fed uncooked liver from the treated calves, 778.3 g/kg for ten rats fed cooked liver from these calves, and 821.8 g/kg for ten rats fed uncooked liver from an untreated calf. None of the rats died, and none fed liver from the treated calves showed signs of intoxication; the prothrombin clotting time was normal in three rats spot-checked at the end of the feeding period. For a 70-kg man, the equivalent of the rats' consumption would be over 6.8 kg of liver a day, an unlikely amount to eat. In addition, humans are much less sensitive to diphenadione than rats; therapeutic doses may exceed 0.5 mg/kg (*8*).

There also appears to be no hazard to calves that consume the milk of treated cows. In one experiment, three cows, each nursing a newborn calf that had no other food source, were given an oral dose (2.75 mg/kg) of diphenadione. There was no change in the plasma prothrombin clotting time of the calves at 12, 24, 48, 72, 96, or 120 hours after the cows had been treated.

Vampire bat control by systemic treatment of livestock with diphenadione has several advantages. First, there is no need for physical contact between the control team and the bats, potential carriers of rabies. Second, only vampire bats that feed on treated animals, a fraction of the total population, are killed. Finally, if area-wide treatments reduce bat populations that feed on livestock more than 90 percent, as our field trials suggest, no more than one or two treatments a year may be necessary. Our tests indicate that relatively high single doses of diphenadione (at least five times the concentration that will make their blood toxic to bats for 3 days) are safe for cattle; in addition, vitamin K is an effective antidote in case of massive overdose. The technique requires that cattle be injected on a body weight basis, but personnel engaged in bat control in countries where vampire bats are a problem are more likely to have experience in this procedure than in handling bats. Since treatments can be administered rapidly, especially if the animals are corralled or lined up in a chute, the technique can be fitted into normal livestock management programs and is applicable on a large scale.

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Curative Potencies against α-Amanitin Poisoning by Cytochrome c

Abstract. *The fatalities occurring after ingestion of the poisonous mushroom* Amanita phalloides *are thought to be due to α-amanitin. Cytochrome c provided antidotal effects against a lethal dose of this toxin in mice. Significant survival was obtained even when the treatment was withheld for 8 hours. The enzyme was superior to a previously explored therapeutic regimen consisting of penicillin and its combination with chloramphenicol and sulfamethoxazole.*

Clinical intoxications resulting from ingestion of the poisonous mushroom *Amanita phalloides* (death cap) are not frequent. In view of the lethality (about 30 percent) (*1*), such cases are dramatic events. Wieland and his collaborators (*2*) have elucidated the chemistry of the poisonous principles. Study of their biological activity (*2, 3*) has revealed that the symptomatology is associated with two main categories of toxins. The rapidly acting phallotoxins, which experimentally cause death in mice within a few hours, are responsible for the gastrointestinal phase of the poisoning. This phase sets in after a latency period of approximately 8 hours and is characterized by diarrhea, nausea, and vomiting. The principal phallotoxin is phalloidin. The amatoxins, which have a 10- to 20-fold greater toxicity, kill mice at lethal doses only after a few days and are thought to cause the late and often fatal hepatorenal phase of the intoxication that sets in after 3 to 5 days. As α-amanitin is quantitatively the most important of the highly toxic amatoxins, it can be assumed to be mainly responsible for the clinical mortality.

No specific treatment of *Amanita* poisoning has been available so far. Several compounds have been recommended on the basis of their alleged clinical effectiveness, but, because of the lack of controlled investigations, the effectiveness could never be substantiated. Also, when tested under controlled conditions, some allegedly useful agents such as thioctic acid, glucocorticosteroids, and coenzyme A did not counteract lethal poisoning with α-amanitin (*4, 5*).

The availability of the pure components of *Amanita phalloides* afforded the opportunity for studying their mode of action. As for α-amanitin, either the liver or the kidney has been shown to be the target organ, depending on the dose (*6*). The poison early induces ultrastructural changes in the nuclei with fragmentation of the nucleoli and segregation of their granular and fibrillar components appearing within only 15 to 30 minutes. Quantities in the range of the minimal lethal dose cause death occurring between 3 and 5 days in mice, with cytoplasmic changes and necrosis of the epithelial cells in the proximal tubules of the kidneys, where the reabsorption of α-amanitin leads to a high concentration of the toxin. With more elevated doses of α-amanitin, the identical damage in the hepatocytes becomes irreversible and causes death at about 2 days. α-Amanitin acts by binding to RNA polymerase in eukaryotic cells and inhibiting this enzyme (*7*). Because of this property, the toxin has been adopted as a tool in molecular biochemical research.

Various agents displaying a protective effect against phalloidin were uncovered, including hepatotoxic compounds such as carbon tetrachloride, thioacetamide, and sodium-cinchophen (*8, 9*). Phenylbutazone and rifampicin also protect against phalloidin (*4*). The first four substances are thought to prevent the transformation of phalloidin to a toxic metabolite by interfering with the function of hepatic microsomal drug-metabolizing enzymes. Carbon tetrachloride and 6-aminonicotinamide have some protective effects against α-amanitin (*9*). Moreover, antamanide, a structural analog of the toxic cyclopeptides, is present in minute quantities in the mushroom and counteracts phalloidin at a suitable dosage (*10*). However, all of the mentioned agents were only effective if given before or at least simultaneously with the toxins. Therefore, the search for antidotes capable of reversing the effects of the toxins at a later stage was pursued but carried out exclusively with α-amanitin.

In a first set of experiments, a series of agents including penicillin, phenylbutazone, chloramphenicol, and sulfamethoxazole were found to counteract lethality if applied after the α-amanitin (*11*). With application as late as 8 hours after the poisoning, a high dose of penicillin or a lower dose of it combined with chloramphenicol and sulfamethoxazole led to survival rates of 30 to 40 percent against the LD_{95} (lethal dose, 95 percent effective) of α-amanitin (0.6 mg/kg). These substances may enhance the excretion of

Table 1. Survival of mice poisoned with α-amanitin and treated with an intraperitoneal injection of cytochrome c. Female white mice (*19*) weighing 18 to 20 g were treated 30 minutes or 8 hours after the intraperitoneal injection of α-amanitin (0.8 mg/kg). The α-amanitin and cytochrome c were dissolved in isotonic saline and administered in a volume of 0.1 ml per 10 g of body weight. The controls received saline (0.1 ml/10 g) only. Penicillin G (sodium benzylpenicillinate) was applied intraperitoneally, chloramphenicol (sodium succinate) subcutaneously, and sulfamethoxazole in water by gavage. Survival of the mice was assessed at 1 week after the administration of α-amanitin. No more fatalities occurred after this time.

Treatment	Dose (mg/kg)	Time after α-amanitin (hours)	No. of mice per group	Survival (%)
Controls			50	0
Cytochrome c	50	½	25	44*
Cytochrome c	50	8	38	34*
Cytochrome c	150	½	19	53*
Cytochrome c	150	8	20	30*
Penicillin-G	1000	8	26	4
Penicillin-G + cytochrome c	1000 150	8	8	62*
Penicillin-G Chloramphenicol Sulfamethoxazole	500 125 500	8	26	8
Penicillin-G Chloramphenicol Sulfamethoxazole + cytochrome c	500 125 500 150	8	8	37*

* The chi-square test, $P < .01$