(Fig. 1) from *Rhizoctonia leguminicola*, which is responsible for producing excessive salivation in cattle (*24*). The occurrence of indolizidine alkaloids in the Leguminosae emphasizes their structural affinity with the pyrrolizidine and quinolizidine alkaloids (*25*). Quinolizidine alkaloids are quite widespread in legumes, and it is possible that indolizidine alkaloids may also occur more frequently in this plant family than has been previously demonstrated.

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20. The soluble material had a melting point of 144° to 146°C (decomposition) and values of optical rotation of $[\alpha]^{25}_{589}$, −84.0°; $[\alpha]^{25}_{578}$, −87.7°; $[\alpha]^{25}_{546}$, −99.2°; and $[\alpha]^{25}_{436}$, −165.4°; ethanol (*c*, 0.99). This substance when mixed with an authentic sample of swainsonine had a melting point of 144° to 146°C. The optical rotation of the authentic sample was measured as $[\alpha]^{24}_{589}$, −83.4°; $[\alpha]^{24}_{578}$, −87.2°; $[\alpha]^{24}_{546}$, −98.4°; and $[\alpha]^{24}_{436}$, −164.7°; ethanol (*c*, 0.32). The chloroform-insoluble alkaloid had a melting point of 161° to 163°C (decomposition).
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22. The ^{13}C NMR spectrum of swainsonine, determined under the same conditions, exhibited four methylene (δ 25.3, 34.6, 54.0, and 62.6 ppm) and four methine (δ 68.3, 71.3, 71.8, and 75.1 ppm) carbon resonances.
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Pregnancy Suppression by Active Immunization Against Gestation-Specific Riboflavin Carrier Protein

Abstract. *A riboflavin carrier protein isolated from chickens cross-reacts with a gestation-specific rodent carrier for riboflavin. Active immunization of female rats of proved fertility with the purified chicken carrier protein completely yet reversibly suppressed early pregnancy without impairing implantation per se. Concurrently there were no discernible adverse effects on maternal health in terms of weight gain, vitamin status, and fertility.*

The mechanism of facilitated transplacental transport and fetal accumulation of riboflavin (and other B group vitamins) in pregnant humans and other mammals (*1, 2*) is unknown, although the relative impermeability of placental membranes to the free vitamin and its coenzyme forms is well recognized (*3*). Recently, we obtained biochemical (*4*) and immunological (*5*) evidence of a gestation-specific high-affinity riboflavin carrier protein (RCP) in the rat. This RCP cross-reacts immunologically with the vitamin carrier in the chicken; in the avian system this vitamin carrier is obligatory for vitamin deposition in the developing oocyte (*6*). The rodent RCP, like its avian counterpart (*7*), is estrogen-inducible and its circulatory concentrations are modulated in concert with changing hormonal levels (*5*). Its importance in fetal development and survival was demonstrated in vivo by passive immunoneutralization which invariably led to acute fetal wastage and abrupt termination of pregnancy (*5*). These observations raised the possibility that immunoneutralization of the RCP in vivo could be exploited as a potentially useful approach to fertility regulation. We now report that active immunization of fertile rats with avian RCP repeatedly terminates early pregnancy with no discernible adverse effects on maternal health in terms of growth, vitamin status, cyclicity, and fecundity.

Part of our evidence for the obligatory participation of RCP in transplacental vitamin transport stems from experiments (*8*) wherein pregnant rats, injected with [^{14}C]riboflavin, were administered a potent and specific antiserum to purified

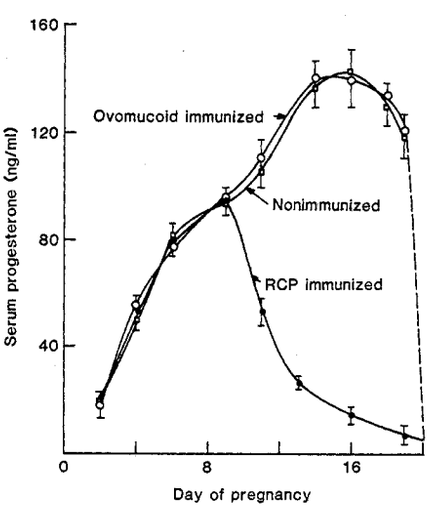

Fig. 1. Effects of active immunization against riboflavin carrier protein and ovomucoid on progesterone concentrations during gestation in rats. The immunization protocol was as described in Table 1. Estrous cycles were evaluated by microscopic examination of vaginal smears. Estrous females were mated with fertile adult males, and the day on which sperms were detected in the vaginal smears was taken as day 1 of pregnancy. Serum prepared from blood withdrawn by cardiac puncture was taken for progesterone estimation by radioimmunoassay (*13*). Serum was deproteinized with methanol and the steroid fraction was extracted with ether. The ether was evaporated off and the steroids were dissolved in buffer (0.01*M* phosphate buffered saline, *p*H 7.4, containing 0.1 percent gelatin). Portions of this were incubated with 1:2000 diluted specific antiserum to progesterone along with ≃ 8000 count/min of [^3H]progesterone (101 Ci/mmole) and then incubated for 3 hours at 0°C; the unbound progesterone was removed by dextran-coated charcoal. The radioactivity of the antibody-bound progesterone was counted.

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chicken RCP to neutralize their endogenous vitamin carrier. After immunoneutralization there was a progressive inhibition (> 95 percent by 18 hours) of fetal uptake of [^{14}C]riboflavin with a concomitant decrease in total flavin content and embryonic weight gain. On the basis of these results we decided to evaluate the effects on rat fertility of active immunization of female rats with heterologous RCP.

To ascertain the long-term effects of the active immunization on maternal health, we repeatedly injected inbred Wistar rats (150 g) of proved fertility with the chicken RCP until their antibody titers were reasonably high as determined by qualitative and quantitative immunochemical methods (*9*). Nonimmunized littermates and littermates actively immunized with ovomucoid [a highly immunogenic chicken protein with no vitamin binding capacity (*10*)] served as controls.

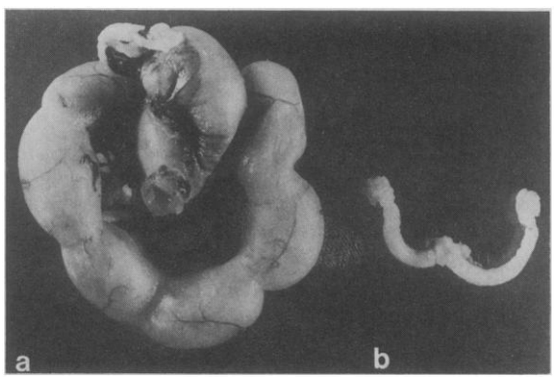

Fig. 2. The relative sizes of uterine horns and inclusions dissected from pregnant rats that were (a) immunized with ovomucoid and (b) actively immunized with chicken RCP. Autopsy was performed on day 18 of pregnancy. The uterine horns in (a) are typical of those from nonimmunized animals as well as animals immunized with ovomucoid.

The total flavin content and glutathione reductase activity of erythrocytes have been used extensively as sensitive biochemical indices of riboflavin status in humans and other animals and for prognosis of vitamin deficiency prior to the appearance of gross clinical manifestations (*11, 12*). We used these parameters as indices of maternal health in our experiments and found that neither growth nor vitamin status of the animals actively immunized with RCP was impaired during the experimental period of 2 to 3 months (Table 1). This indicates that the gestation-specific vitamin carrier has no essential physiological function in maternal vitamin nutrition and well-being. We also found that the vitamin carrier could not be detected by specific radioimmunoassay in adult male rats, ovariectomized females, or rapidly growing immature animals (*5*). Since the immunized females maintained their regular 4-day estrous cycle, it appears that high titers of RCP antibodies have no adverse influence on their reproductive hormonal profiles.

When such animals were mated with fertile adult males they conceived normally, as was apparent from the number of implantation sites in their uterine horns at laparotomy on day 7 of pregnancy. However, when the pregnancies were monitored by assaying (*13*) circulating progesterone as an index of ovarian and placental function (*14*), the initial linear increase in the serum progesterone concentration abruptly reversed after day 9 [that is, soon after the placenta became functional (*15*)], signaling fetal death or resorption (*16, 17*) (Fig. 1). This is in marked contrast to the pattern in the ovomucoid-immunized and nonimmunized control animals, in which progesterone continued to increase and remained elevated until day 18 when it began to slowly decrease (*14*), indicating uninterrupted pregnancy till term. Laparotomy on day 18 confirmed these observations. Figure 2 shows uterine horns containing well-grown fetuses typical of the control and ovomucoid-immunized animals compared with the excised uteri from RCP-immunized animals. The latter are devoid of any detectable fetal tissue and are indistinguishable from the uteri of nonpregnant animals.

As shown in Table 2, the effective

Table 1. The effect of active immunization with RCP and ovomucoid on body weight gain and riboflavin status of female rats. Female rats (150 g) were injected intradermally at weekly intervals for 6 weeks with purified chicken RCP or ovomucoid (50 μg per animal each time) emulsified with Freund's complete adjuvant. The specificity of the antibodies was detected by immunodouble diffusion and immunoelectrophoresis on agarose and measured by quantitative immunoprecipitation (*9*). The antiserum (diluted 1:100) from RCP-immunized animals specifically bound ^{125}I-labeled RCP, whereas antiserum from ovomucoid-immunized animals did not. Both sets of antiserum combined with about 400 μg, per milliliter, of the respective antigens at the equivalence point. Total flavin content of deproteinized erythrocyte hemolyzates was estimated fluorimetrically by measuring fluorescence (reducible by sodium hydrosulfite) emitted at 530 nm on excitation at 470 nm (*11*). The glutathione reductase activity of the erythrocytes was quantified in the presence and absence of flavin adenine dinucleotide (FAD) (3 μmole) as described elsewhere (*12*).

Group	Number of animals	Weight gain (g)*	Erythrocyte glutathione reductase activity*†		Erythrocyte flavin content (ng/ml blood)
			−FAD	+FAD	
Nonimmunized	4	61 ± 16	540 ± 30	515 ± 44.37	88.2 ± 12.74
Ovomucoid immunized	5	64 ± 28	544 ± 11.16	508 ± 23.00	74.2 ± 15.70
Chicken RCP immunized	10	65 ± 16	525 ± 24.77‡	518 ± 22.61‡	83.3 ± 11.80‡

*Mean ± standard deviation. †Expressed as micrograms of reduced glutathione formed per milliliter of blood per 15 minutes at 37°C. ‡Not statistically significant compared to either nonimmunized controls or ovomucoid immunized animals.

Table 2. The influence of active immunization with RCP or ovomucoid on pregnancies in female rats. The immunization protocol was as described in Table 1. The number of implantation sites before and after immunization was determined at laparotomy on day 7 of pregnancy.

Group	Number of animals	Implantation sites before immunization		Remarks	Implantation sites after immunization		Remarks	Weight gain as on day 18 of pregnancy* (g)
		Total	Average per animal		Total	Average per animal		
Nonimmunized	5	49	9.8	Normal pups	46	9.2	Normal pups	98 ± 14
Ovomucoid immunized	5	45	9.0	Normal pups	48	9.6	Normal pups	81 ± 11
Chicken RCP immunized	12	119	9.9	Normal pups	103	9.42	No pups†	9 ± 6.0

*Mean ± standard deviation. †No implantation sites were evident at day 20 of pregnancy; slight vaginal bleeding occurred on day 11 of pregnancy.

neutralization of endogenous RCP invariably terminated early pregnancies without curtailing implantation per se. The marginal weight gain in the RCP-immunized animals contrasts with the marked weight increases in the nonimmunized and ovomucoid-immunized animals during 18 days of gestation (Table 2) and reflects early embryonic loss in the RCP-immunized rats. Thus, it appears that physiological processes and endocrine functions vitally concerned with ovulation, fertilization, tubal transport, and subsequent blastocyst formation and implantation were not impaired by active immunization. After termination of early pregnancies the RCP-immunized animals resumed their 4-day estrous cycles and conceived normally when mated. Again there was no apparent interference with the process of implantation, but from day 9 of pregnancy the serum progesterone levels sharply declined indicating fetal death. This pattern of early interference with pregnancy could be demonstrated repeatedly as long as high antibody titers were maintained by booster injections of the antigen. If, however, the titer was allowed to wane with lapse of time (3 months after a booster injection), the animals carried their pregnancies to term and delivered pups with no detectable abnormality.

These findings not only demonstrate the reversibility of the effects of active immunization with RCP, but also imply that antibodies to the heterologous vitamin carrier, though capable of effectively neutralizing the endogenous RCP (and hence interfering with embryonic development), nonetheless fail to boost significantly the animals' immunological memory to render the process irreversible for prolonged periods. This is reminiscent of the situation pertaining to fertility regulation in subhuman primates (*18*) actively immunized with the β-subunit of human chorionic gonadotropin and in women administered the hormonal subunit coupled to tetanus toxoid (*19*).

These data indicate that the vitamin carrier protein is obligatory for transplacental riboflavin transport and supply to the developing fetus in higher animals. Similar proteins have been discovered in pregnant cows (*20*) and primates (*5*). It would thus be interesting to determine whether active immunization against the pregnancy-specific vitamin carrier could also terminate early pregnancy in primates.

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Abnormal Glutamate Metabolism in an Adult-Onset Degenerative Neurological Disorder

Abstract. *In patients with recessive, adult-onset olivopontocerebellar degeneration associated with a partial deficiency of glutamate dehydrogenase, the concentration of glutamate in plasma was significantly higher than that in controls. Plasma α-ketoglutarate was significantly lower. Oral administration of monosodium glutamate resulted in excessive accumulation of this amino acid in plasma and lack of increase in the ratio of plasma lactate to pyruvate in the glutamate dehydrogenase–deficient patients. Decreased glutamate catabolism may result in an excess of glutamate in the nervous system and cause neuronal degeneration.*

Degenerative neurological disorders, such as Huntington's chorea, Parkinson's disease, and spinocerebellar ataxia, are all characterized by selective, premature loss of nerve cells. The pathogenesis of this neuronal degeneration has been the subject of intense investigations, but remains unknown. In recent years considerable interest has been aroused by observations showing that certain neuroexcitatory amino acids, such as glutamate and its potent analogs kainic acid and ibotenic acid, are capable of producing selective nerve cell destruction while sparing other elements of nervous tissues (*1*). Furthermore, when these compounds are injected into certain brain areas of experimental animals, they produce morphological and biochemical alterations found in patients with degenerative neurological disorders (*1*).

Although these findings have raised the possibility that similar neurotoxic substances accumulate in human nervous tissues and cause neuronal degeneration, evidence for this has been lacking. In the present study we investigated patients with a genetic neurological disorder known as olivopontocerebellar atrophy (OPCA). The disease, a form of which is associated with a partial deficiency of glutamate dehydrogenase (GDH) (E.C. 1.4.1.3), affects adults and is characterized by progressive atrophy of areas of the brainstem, cerebellum, spinal cord, and substantia nigra and by ataxia, corticospinal deficits, dysarthria, dysphagia, and signs of parkinsonism (*2*). It is regarded as the classical structural neurological disorder linking the spinocerebellar degenerative conditions with the extrapyramidal diseases. We found decreased glutamate catabolism in patients with this condition. The accumulation of glutamate in the nervous tissue may well be the cause of the neuronal degeneration.

In previous studies, we showed that leukocytes and cultured skin fibroblasts from patients with a recessive form of OPCA have decreased GDH activity (*3*). The enzymatic deficiency was identified when several enzymes requiring nicotinamide adenine dinucleotide phosphate [NAD(P)] were evaluated in the fibroblasts. The rationale for these investigations was based on observations of the neurotoxic effects of the nicotinamide antagonist 3-acetylpyridine in the rat (*3*).

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