The above reported activity of 6β-hydroxy-Δ¹THC may complicate still further the quest toward the understanding of the molecular basis of *Cannabis* activity. Possibly Δ¹THC is likewise metabolized by humans to two (or more) active metabolites. This stipulated parallelism to rabbit liver homogenate metabolism, though plausible, has yet to be confirmed. If this is indeed the case, it will be of considerable importance to determine whether the nonidentical effects of *Cannabis* on different individuals are due, in part at least, to different ratios of Δ¹THC and metabolites (or only metabolites) at the receptor sites. Possibly each of these compounds acts in a slightly different fashion. Such a phenomenon is to be expected. The few psychotomimetically active cannabinoids that have so far been administered to humans cause effects that are by no means identical to those of Δ¹THC (*13, 14*).

It is generally assumed that the nonidentical psychological reactions produced by *Cannabis* are due to the different personalities of the users and the varying environmental conditions. While these factors are probably of considerable importance, one has now to take into account the possibility that several compounds (in varying ratios and with presumably different biochemical profiles) contribute to the overall effects.

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Galactose Toxicity in the Chick: Hyperosmolality

Abstract. *Galactose-fed chicks have been found to develop severe hyperosmolar dehydration. Although biochemical abnormalities have been observed in the brain of the galactose-toxic chick, the observed physiologic alteration of serum osmolality could be the major factor responsible for the galactose toxicity syndrome in the chick.*

The ingestion of galactose by chicks results in a syndrome consisting of shivering, generalized motor seizures, debilitation, and eventual death. The etiology of this syndrome is an enigma, but it has attracted attention as an animal model for the study of biochemical alterations leading to the mental retardation that occurs in galactosemia. It is known that the Rhode Island Red is more sensitive to galactose feeding than the Leghorn (*1*) and that the female is more sensitive than the male. Paradoxically, estrogen increases, whereas testosterone decreases, resistance to galactose feedings. In addition, galactose sensitivity decreases as the age of the animal increases (*1*). The accumulation of galactose metabolites in the tissues has been reported (*2, 3*), despite the fact that the galactose-fed chick is capable of metabolizing significant quantities of galactose (*4*). The clinical manifestations and the evidence of accumulated metabolites cease within 24 hours after galactose is removed from the diet (*2*).

Thus far, investigation has focused on a biochemical explanation for these observations. While working with galactose-fed chicks, however, we were struck by the severity of the animals' debilitation. The experimental animals are lighter and less vigorous than age-matched controls; when killed, the experimental animal bleeds very little. We also have observed that the experimental animal excretes large amounts of watery waste material not seen in control animals. Since these results suggest alteration in body fluids and dehydration, the galactose-toxic chick was investigated for changes in serum osmolality.

One-day-old Rhode Island Red chicks were obtained from Hall Brothers' Hatchery, Wallingford, Connecticut. Control animals were fed a diet of nonmedicated chick feed and water ad libitum. Experimental animals were given 10 percent galactose (weight-volume) in their drinking water for 2 days prior to being killed. Glucose and galactose concentrations in blood and cloacal contents were determined on protein-free filtrates as the trimethylsilyl ether derivative by gas-liquid chromatography (*2*). Serum osmolality was determined on an osmometer (Fiske model 300).

One-day-old chicks fed 10 percent galactose in their drinking water for 2 days have large amounts of galactose in their blood and cloacal contents (Table 1). The serum osmolalities of these chicks are approximately 180 milliosmoles per kilogram greater than those of control chicks of the same age. Serum osmolalities 100 milliosmoles per kilogram greater than those of control animals persist up to 5 days of age. It is during this 3- to 5-day period when sensitivity to galactose appears to be greatest, and when the greatest percentage of fatalities occurs (*1, 2*).

Table 1. Comparison of glucose and galactose concentrations and serum osmolality in both control and galactose-fed chicks. One-day-old male and female chicks were each fed 10 percent galactose for 2 days prior to being killed. Glucose and galactose concentrations in the blood and cloacal contents were determined on protein-free filtrates as described in the text. The serum osmolality was determined by the freezing-point depression. Values are the mean ± standard error of the mean. Numbers in parentheses indicate the number of individual determinations.

Age when killed (days)	Animal	Weight (g)	Blood		Cloacal contents		Serum osmolality (milliosmole/kg)
			Glucose (mg/100 ml)	Galactose (mg/100 ml)	Glucose (mg/g)	Galactose (mg/g)	
3	Control	37.8	250 ± 14 (4)	None	Nondetectable		300 ± 4 (4)
3	Galactose-fed	32.9	233 ± 19	1272 ± 212 (6)	Trace	64*	484 ± 38 (7)
4	Control	38.9					304 ± 4 (6)
4	Galactose-fed	33.4					410 ± 19 (5)
5	Control	43.6	252 ± 17 (4)	None			309 ± 2 (8)
5	Galactose-fed	39.3	307 ± 22	964 ± 89 (6)			427 ± 32 (8)
7	Control	50.2	254 ± 19 (5)	None	Nondetectable		310 ± 3 (5)
7	Galactose-fed	47.7	277 ± 13	866 ± 84 (6)	Trace	19*	386 ± 12 (5)

* Values are the means of duplicate determinations.

Chicks 7 days of age which had been fed galactose from 4 to 5 days of age showed serum osmolalities about 70 milliosmoles per kilogram greater than those of 7-year-old controls. Table 2 shows that females have serum osmolalities 130 milliosmoles per kilogram greater than those of the males after 2 days of galactose feeding. Removal of galactose from the diet results in the return of serum osmolality to normal values in 24 hours (Table 2) and cessation of all clinical manifestations of galactose toxicity.

The serum osmolalities of chicks fed 10 percent galactose for 2 days demonstrate a previously unrecognized hyperosmolar dehydration (Table 1). The high concentration of galactose in the cloacal contents suggests an osmolar diuresis as the mechanism. Whatever the cause, the hyperosmolality could be responsible for the entire "toxicity syndrome." As seen in Table 1, the normal chick gains approximately 3 g a day. The serum osmolality in older chicks fed galactose decreases with the increasing starting weight of the animal (Table 1). Feeding galactose to a 7-day-old chick resulted in no toxicity syndrome at the concentrations of galactose utilized in this study. It appears that, as the chick gains weight, it is less susceptible to dehydration. This may be due to increased organ mass which increases the amount of galactose metabolized, leaving a smaller percentage for excretion and obligate water loss.

The shaking and seizures can be explained to be the result of serum hyperosmolality. Focal seizures have been reported in humans (*5*, *6*) with hyperosmolar hyperglycemia. Infusions of glucose into normal dogs to a concentration of 2000 mg per 100 ml produced restlessness, nystagmus, and seizures (*7*). The mechanism of the seizures in these cases is uncertain. It has been postulated that the cause may be intracellular dehydration and disturbances of electrolyte equilibrium of the intra- and extracellular compartments (*6*). The severe dehydration indicated by the serum osmolalities found in galactose-fed chicks makes this a possible explanation for the neurologic manifestations. The immediate reversibility of the syndrome when galactose is removed from the diet suggests a transient phenomenon which supports dehydration as the mechanism for the neurologic syndrome.

The increased sensitivity of the female chick to galactose does not seem to be related to size, inasmuch as no significant weight difference was found between the sexes. However, female chicks as a group have higher serum osmolalities after 2 days of galactose feeding than male chicks. This difference may be due to impaired galactose metabolism in the female as a result of the lower concentrations of galactose-I-phosphate uridyltransferase in the female liver (*8*). Nordin *et al.* (*1*) observed the paradox of the more galactose-sensitive female chick being protected from galactose toxicity by estrogens and being made more sensitive to galactose with testosterone. This result can be explained in terms of weight. In the initial description of this phenomenon the observation was made that diethylstilbestrol enhances, whereas testosterone depresses, the growth of chicks (*1*). Thus, it follows that the enhanced growth produced by estrogen was protective in that it increased the animals' size and capacity to metabolize galactose.

The galactose toxicity syndrome of the chick has been thought to be an exaggeration of the more subtle abnormalities seen in human galactosemia. Several biochemical abnormalities have been postulated as a mechanism for this toxicity syndrome. Decreased turnover of phospholipids (*9*) and glycoproteins (*10*) in the presence of normal pools of these substances have been observed. Lower brain concentrations of adenosine triphosphate also have been reported (*9*), but in our laboratory, using the minimum amount of galactose in the feed necessary to produce neurotoxicity, we have shown the concentrations of adenosine triphosphate to be normal (*11*).

The significance of these biochemical abnormalities and their relationship to galactose per se now seems to fade in

Table 2. Serum osmolality of control and galactose-fed chicks. One-day-old male and female chicks were each fed 10 percent galactose in their drinking water for 2 days; several of each group were killed 1 day later. Surviving chicks were given water instead of galactose solution. Some animals were killed at 24 hours and some at 48 hours after the removal of galactose from the diet. Control animals, comparable in age to the galactose-fed chicks, were given water throughout the experiment. Values are the mean ± the standard error of the mean and are expressed as milliosmoles per kilogram. Numbers in parentheses indicate the number of individual determinations.

Age when killed (days)	Control	Galactose-fed females	Galactose-fed males
3	306 ± 2 (10)	523 ± 31 (10)	390 ± 18 (9)
	At 24 hours after galactose was removed from the diet		
4	304 ± 3 (8)	307 ± 3 (4)	306 ± 4 (4)
	At 48 hours after galactose was removed from the diet		
5	308 ± 3 (8)	310 ± 3 (6)	302 ± 4 (6)

view of the hyperosmolality reported here and the complete reversibility of the syndrome within 24 hours after galactose is removed from the diet. The hyperosmolar dehydration and its consequent effect on the perfusion of organs make the earlier biochemical observations seem rather nonspecific and suggest that the galactose-fed chick may be a better model for the study of hyperosmolar neurotoxicity than for the study of galactose neurotoxicity.

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Prenatal Cerebral Development: Effect of Restricted Diet, Reversal by Growth Hormone

Abstract. *Caloric restriction of rats from day 10 to day 20 of pregnancy results in significant decreases in body weight, placental weight, cerebral weight, cerebral DNA, and cerebral protein of the offspring at birth. These decreases did not usually occur if mothers on the restricted diet were treated concomitantly with bovine growth hormone. If growth hormone did not cross the placenta, then, it is postulated, at least one effect of growth hormone was the mobilization of maternal nutrient reserves.*

Bovine pituitary growth hormone, when administered to tadpoles, produces significant increases in cerebral cell number and cerebral DNA (*1*). When administered to pregnant rats, this hormone was reported to increase neonatal cerebral weight and cerebral DNA (which is proportional to cell number) (*2*); these results, however, varied considerably, and the effect on rat cerebral DNA in particular was often diminished (*3*).

Engfeldt and Hulquist (*4*) reported that the effect of growth hormone on neonatal body weight can be demonstrated only for mothers and offspring of low body weight. This suggested to us that the effect of growth hormone on prenatal brain development may be more pronounced if the pregnant rats are nutritionally restricted. Because of the known involvement of growth hormone in carbohydrate metabolism, and the importance of glucose as energy source for the fetus, caloric restriction was investigated first.

We have maintained pregnant rats on a diet with one-third of the normal caloric value (*5*) but with protein and vitamin content identical to that in the diet for the control group. In this respect our diet differs from those in earlier experiments, in which total food intake was reduced and protein and vitamin restriction were produced as well as caloric restriction. Before the start of caloric restriction on day 10 of pregnancy, the diet of restricted and control animals was the same. During the 10 days on the restricted diet, the daily food intake of restricted animals contained 2.4 g of protein, 0.8 g of carbohydrate, and 20.3 kcal. During this 10-day period the daily food intake of the control animals contained 2.8 g of protein, 7.1 g of carbohydrate, and 56.5 kcal. Except for diet, the experiment was similar to our earlier protein restriction experiments (*6, 7*).

The females in group R (Table 1) were maintained on the low calorie diet from day 10 to day 20 of pregnancy. The animals in group R + GH were also maintained on this diet and concomitantly received daily intravenous injections of bovine growth hormone (*8*) (3 mg in 1 ml of saline, adjusted to *p*H 7.4). The peak concentration of growth hormone in blood was estimated to be 4000 times the natural value in fasting rats; the total dose (30 mg) was estimated to be 100 times the total content of growth hormone in the pituitary of an adult female rat (*9*). Natural increases in hormone, for example those

Table 1. The effect of maternal treatment with bovine growth hormone on neonatal offspring. Abbreviations are as follows: R, restricted diet (one-third of the number of calories of the control diet) administered from day 10 to day 20 of pregnancy; C, control diet (pelleted food ad libitum); GH, bovine growth hormone administered 3 mg/day intravenously, concomitantly with restricted diet (*8*); Δ, percent difference; Δ_C, percent difference from group C; Δ_R, percent difference from group R. Standard deviations follow each weight.

Group	Mothers	Offspring	Body		Placenta		Cerebrum					
							Total		DNA		Protein	
			Weight (g)	Δ (%)	Weight (g)	Δ (%)	Weight (g)	Δ (%)	Content (μg)	Δ (%)	Content (mg)	Δ (%)
R	11	97	4.0 ± 0.8		0.395 ± .078		0.131 ± .015		539 ± 35		7.06 ± 0.84	
Δ_C				− 33*		− 24*		− 19*		− 11*		− 17*
R + GH	8	58	4.9 ± 0.5		0.478 ± .090		0.159 ± .012		588 ± 39		8.18 ± 0.93	
Δ_C				− 18*		− 8		− 2		− 3		− 4
Δ_R				+ 23*		+ 21*		+ 21*		+ 9*		+ 16*
C + GH	5	55	6.2 ± 0.6		0.516 ± .105		0.176 ± .018		616 ± 28		9.40 ± 0.62	
Δ_C				+ 3		+ 1		+ 8*		+ 1		+ 10*
C	10	59	6.0 ± 0.4		0.520 ± .071		0.162 ± .013		607 ± 35		8.51 ± 0.66	

* $P < .001$.