Volz rejected the analysis by Dyer and Hicks (*2*) because "optical soundings of the stratosphere indicated no stratospheric dust amounts and no seasonal variations of such magnitude" (*7*) and because Dyer and Hicks failed to detect an increase in stratospheric dust in summer after the Mount Agung eruption (*8*). However, less violence is done to the literature on this subject by rejecting Volz's (*7*) data for the Northern Hemisphere since they reveal neither the general rise in optical thickness after the Mount Agung eruption (*1*, *4*) nor its seasonal fluctuations (*4*, *9*).

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References and Notes

1. H. T. Ellis and R. F. Pueschel, *Science* **172**, 845 (1971).
2. A. J. Dyer and B. B. Hicks, *Quart. J. Roy. Meteorol. Soc.* **94**, 545 (1968).
3. E. C. Flowers, R. A. McCormick, K. R. Kurfis, *J. Appl. Meteorol.* **8**, 955 (1969).
4. F. E. Volz, *J. Geophys. Res.* **75**, 1641 (1970); F. Burdecki, *Weather* **1964**, 113 (1964).
5. W. M. Irvine and F. W. Peterson, *J. Atmos. Sci.* **27**, 62 (1970).
6. Most unambiguously documented in the case of O_3 but also apparent in measurements of stratospheric H_2O and dust [see (*4*, *5*)].
7. F. E. Volz, *J. Geophys. Res.* **75**, 5185 (1970).
8. ———, personal communication.
9. G. Grams and G. Fiocco, *J. Geophys. Res.* **72**, 3523 (1967).
10. Performed under the auspices of the U.S. Atomic Energy Commission.

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We have subjected the data from Mauna Loa Observatory to a harmonic analysis with the following results: (i) Since about 1970, the annual average amount of atmospheric turbidity is the same as it was prior to 1963; (ii) monthly averages of turbidity data for the period 1970–1971 agree in amplitude and phase with the annual periodicity observed for 1958–1962; (iii) the annual average as well as the amplitude of the periodic turbidity function are significantly reduced for a 5-year period of strong volcanic activity (*1*) following the eruption of Mount Agung in March 1963.

These results substantiate what we reported earlier (*2*). In particular, they contradict the finding by Dyer and Hicks (*3*) of an increased annual turbidity amplitude after 1963, which is the basis of Ellsaesser's argument that the seasonal turbidity fluctuations observed on Mauna Loa reflect changes in the stratospheric aerosol load. Confined to the stratosphere or troposphere, these fluctuations apparently are characteristic of the variations in the atmospheric background aerosol. Any perturbation in the amplitude or phase of the annual periodicity, or both, such as was the case during 1963–1969, are indicative of a change in the colloidal composition of the atmosphere. We wish to emphasize again that such perturbations should be utilized to evaluate man's impact on our atmospheric environment.

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References

1. J. F. Cronin, *Science* **172**, 847 (1971).
2. H. T. Ellis and R. F. Pueschel, *ibid.*, p. 845.
3. A. J. Dyer and B. B. Hicks, *Quart. J. Roy. Meteorol. Soc.* **94**, 545 (1968).

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Galactose Toxicity in the Chick: Hyperosmolality or Depressed Brain Energy Reserves?

In a recent report Malone *et al.* (*1*) described the close correlation of hyperosmolality with elevated galactose concentrations in the serum of chicks fed water containing 10 g of galactose per 100 ml. Since observations were made over a period during which galactose exerted an extremely toxic effect on the chicks and during which high rates of mortality occurred (3 to 5 days after the initiation of the experiment), Malone *et al.* stated that severe hyperosmolar dehydration could be responsible for the entire galactose toxicity syndrome. However, we have concluded, as a result of recent experiments, that hyperosmolality per se is not the major factor responsible for the galactose toxicity syndrome in the chick. We have observed both physical and biochemical recovery from the neurotoxicity upon elevation of blood glucose concentrations. On the basis of previous studies (*2–4*) and evidence presented here, we propose that galactose interferes with the supply of glucose to the brain, thus affecting brain energy metabolism.

Two-day-old male Leghorn chicks were obtained from Klager Hatcheries, Bridgewater, Michigan and were maintained in a brooder at 32°C. The animals were fed a semisynthetic diet (*5*), 40 percent (by weight) of which was replaced with D-galactose at the expense of Cerelose. Osmolality was determined with an osmometer (Fiske G-66) on plasma samples obtained by heart puncture. Both preparation and analysis of brain metabolites were performed as previously described (*2*).

After chicks had been fed their respective diets for 48 hours (control diet, group A; galactose diet, groups B, C, and D), animals in group C were injected intraperitoneally with 1 ml of a 1*M* D-glucose solution and those in group D were injected intraperitoneally with 1 ml of 0.5*M* NaCl solution. The chicks in group C no longer exhibited convulsions and tremors, whereas the appearance of those chicks in group D was unchanged. The duration of the physical recovery correlated with an increase in the concentrations of plasma glucose (Table 1). Similarly, during the recovery phase, the brain concentrations of adenosine triphosphate (ATP), phosphocreatine, glucose, fructose-1,6-diphosphate, and lactate returned virtually to normal values (Table 2, group C), whereas injection of saline had essentially no effect on the concentrations of these metabolites (Table 2, group D).

As previously reported (*2*, *4*), the inclusion of galactose in the diet resulted in depressed concentrations of brain glucose and glucolytic intermediates (Table 2, group 8) without affecting the blood glucose concentrations (Table 1, group B). However, when the plasma glucose concentration was significantly elevated, the concentrations of brain metabolites approached those of normal chicks. Thus, these experiments support our contention that galactose interferes with glucose transport across the blood brain interface and that this interference may be the major cause of the neurotoxicity in chicks.

The suggestion by Malone *et al.* (*1*) that hyperosmolality may be the leading factor in galactose neurotoxicity in the chick cannot be inferred from our experiments. Osmolalities were determined on plasma simultaneously analyzed for glucose and galactose concentrations, and are listed in Table 1. Severely disabled chicks (group B) had plasma osmolality values (in milliosmoles per kilogram) of 335 ± 10 as compared with 309 ± 7 for controls. The injection of glucose, which temporarily reversed the neurotoxicity, further elevated the osmolality by 12 (347 ± 7) after 20

Table 1. Comparison of plasma osmolality and galactose and glucose concentrations during a period of galactose toxicity in the chick. Plasma galactose and plasma glucose concentrations were determined on protein-free filtrates by methods in which glucose oxidase or galactose oxidase were used (*8*). Values represent the mean ± the standard deviation for four or five pools, each containing either four or five chicks.

Group	Time after injection* (minutes)	Plasma osmolality (milliosmole/kg)	Plasma concentration (mg/100 ml)	
			Galactose	Glucose
A		309 ± 7	None	253 ± 15
B		335 ± 10	503 ± 21	259 ± 15
C	20	347 ± 7	442 ± 48	644 ± 90
C	60	338 ± 11	499 ± 88	393 ± 32
C	240	323 ± 4	310 ± 35	283 ± 18
D	20	352 ± 9	517 ± 150	260 ± 28
D	60	348 ± 6	414 ± 52	261 ± 24
D	240	342 ± 9	234 ± 54	259 ± 13

* Chicks in groups A and B received no injections; chicks in group C were injected intraperitoneally with 1 ml of 1*M* D-glucose solution; chicks in group D were injected intraperitoneally with 1 ml of 0.5*M* NaCl solution.

Table 2. Comparison of the concentrations of selected brain metabolites during the period of galactose toxicity in the chick. Experimental conditions and animal designations are the same as those described in Table 1 and in the text. Values represent the mean ± the standard deviation for three pools, each containing either four or five chicks.

Group	Time after injection (minutes)	Brain metabolite (μmole/g)				
		ATP	Phospho-creatine	Glucose	Lactate	Fructose-1,6-diphosphate
A		1.90 ± 0.10	1.87 ± 0.15	1.5 ± 0.4	3.3 ± 0.3	0.16 ± 0.02
B		1.68 ± .10	1.66 ± .11	0.3 ± .1	1.7 ± .5	.04 ± . 01
C	20	1.88 ± .20	2.33 ± .20	1.2 ± .4	2.6 ± .2	.14 ± .04
C	60	1.81 ± .06	2.16 ± .15	1.3 ± .6	2.9 ± .7	.10 ± .02
C	240	1.60 ± .17	1.65 ± .17	0.3 ± .1	1.5 ± .2	.03 ± .01
D	20	1.67 ± .14	1.78 ± .20	0.3 ± .1	1.7 ± .1	.05 ± .01
D	60	1.66 ± .20	1.66 ± .18	0.3 ± .1	1.7 ± .4	.04 ± .01
D	240	1.58 ± .13	1.54 ± .11	0.4 ± .1	1.7 ± .1	.05 ± .01

minutes. As expected, the group injected with a hypertonic NaCl solution (Table 1, group D) showed an elevated osmolality (352 ± 9) 20 minutes after injection, but virtually no change in the severity of the neurotoxicity.

In our studies, severe toxicity was observed when the plasma galactose concentration was 503 ± 21 mg/100 ml and the osmolality was 335 ±10 milliosmole/kg, values distinctly lower than those reported by Malone *et al.* (*1*) (range of plasma galactose concentration, 866 to 1272 mg/100 ml; range of osmolality, 386 to 484 milliosmole/kg). These differences are probably the result of feeding galactose in the form of a semisynthetic diet with free access to pure water rather than in the form of a hyperosmolar (0.56*M*) galactose solution (10 percent) for drinking water.

Our studies confirm the observation of Malone *et al.* (*1*) that hyperosmolality accompanies hypergalactosemia. However, according to our studies, osmolality appears to be a nonspecific factor in galactose toxicity, and we suggest that measurements of plasma osmolality correlate better with galactose concentrations of galactose-fed chicks than with the neurotoxicity symptoms. It is our view from numerous studies of this model (*2–4*, *6*) that galactose neurotoxicity in the chick involves a complex balance of energy requirements versus the availability of energy reserves (*7*).

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References and Notes

1. J. I. Malone, H. J. Wells, S. Segal, *Science* **174**, 952 (1971).
2. L. P. Kozak and W. W. Wells, *Arch. Biochem. Biophys.* **135**, 371 (1969).
3. ———, *J. Neurochem.* **18**, 2217 (1971).
4. S. E. Granett, L. P. Kozak, J. P. McIntyre, W. W. Wells, *ibid.*, in press.
5. W. J. Rutter, P. Krichevsky, H. M. Scott, R. G. Hansen, *Poultry Sci.* **32**, 706 (1953).
6. J. C. Blosser and W. W. Wells, *J. Neurochem.* **19**, 69 (1972).
7. H. M. Kalckar, in *Molecular Basis of Some Aspects of Mental Activity*, O. Walaas, Ed. (Academic Press, New York, 1966), vol. 1, p. 223.
8. H. U. Bergmeyer and E. Bernt, in *Methods of Enzymatic Analysis*, H. U. Bergmeyer, Ed. (Academic Press, New York, 1963), p. 123.
9. Supported by PHS grant AM 10209.

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Pinpointing the exact etiology of galactose neurotoxicity in the chick as well as analogous neurological disorders resulting from nutritional manipulation is fraught with difficulty. One may observe many biochemical alterations and make correlations, but it is difficult to determine whether a single event or concatenation of events underlies the toxicity. As a result of many biochemical analyses of the brains of galactose-fed chicks, markedly elevated concentrations of galactose (*1*, *2*), galactose-1-phosphate (*2*), and galactitol (*1*), as well as decreased concentrations of glucose and glycolytic intermediates, have been reported (*2*). Kozak and Wells have observed decreased concentrations of adenosine triphosphate (ATP) and creatine phosphate, and have focused on the decreased ATP concentration and energy change as a primary event in the neurotoxicity (*2*, *3*). In our own biochemical evaluation we were struck by the hyperosmolality induced in chicks by galactose ingestion and indicated that the biochemical abnormalities might be secondarily related to the as yet poorly understood hyperosmolar coma syndrome. Now Knull *et al.* ask us to choose between hyperosmolality and depressed brain energy as the etiology of the syndrome, on the basis of the fact that seizures can be prevented if the blood glucose concentration is restored to the normal value. We do not believe that this complex disorder can be profitably explained by focusing solely on one aspect of brain metabolism, inasmuch as energy availability and hyperosmolality may be closely interrelated in the economy of brain functioning and therefore should not be viewed as mutually exclusive. For example, the hyperosmolality could be contributing to the low glucose concentration in the brain by decreasing brain perfusion as a consequence of dehydration.

We have made two pertinent observations. First, despite the fact that Kozak and Wells reported a marked depletion of ATP in the brains of galactose-toxic chicks and postulated a "futile" adenosine triphosphatase activity (*2*, *3*), we have found no alteration in ATP concentrations in chick brains frozen immediately (1.90 ± 0.02 μmole/g, ten controls; 1.8 ± 0.05 μmole/g, ten galactose-toxic chicks). Knull *et al.* now confirm our observations of no major change in ATP or creatine phosphate concentrations. Indeed, we are confronted with the anomaly of a postulated depletion of energy with no change

in the concentrations of the compounds related to energy stores.

Second, we have substantiated the report of Kozak and Wells (*2*) that the concentration of glucose in the brains of galactose-toxic chicks is lower than that in the brains of normal chicks (Table 1). Indeed, Kozak and Wells, as well as Segal, have previously suggested that galactose interferes with the entry of glucose into the brain (*2*, *4*). Table 1 also demonstrates decreased glucose entry into the brain and shows that, within 10 minutes after the injection of radioactive glucose into normal and galactose-toxic chicks, only half as much of the label is present in the brains of normal chicks as compared with the brains of galactose-toxic chicks. The distribution of label in free brain glucose and glucose metabolites appears to be identical in both groups of animals. In view of the known sharing of glucose and galactose transport systems in various tissues, our first inclination is to propose that galactose transport competes with glucose transport. Decreased brain perfusion in galactose-toxic animals could also produce the same result.

Knull *et al.* raise the question of differences in the technique of galactose administration. In their various experiments Kozak and Wells used two types of administration, a 40 percent galactose feed and 10 percent galactose in drinking water, the latter method being identical to our method. In both cases they reported identical findings (*2*). In their early experiments with galactose-toxic chicks Nordin *et al.* gave a 15 percent galactose feed and found the Rhode Island Red strain to be more sensitive than the Leghorn (*5*). We have used a 5 percent galactose solution as the drinking water for the Rhode Island Red strain, but we found that this concentration was inadequate to consistently produce the syndrome and we therefore had to resort to a 10 percent solution to produce the desired effects. The variation in blood galactose concentration and osmolality in different experiments may, however, be related more to the time of animal sacrifice after galactose ingestion than to the means of galactose administration.

Table 1. Uptake of radioactivity in the brain after intracardiac injection of uniformly labeled [^{14}C]glucose. Chicks were decapitated into liquid nitrogen 5 minutes after intracardiac injection. Aliquots of frozen brain and plasma were solubilized in Soluene, and the radioactivity of each fraction was counted. The radioactivity of the plasma was 320,000 counts per minute per milliliter in both control and galactose-fed chicks. The radioactivity per milliliter of brain water was calculated on the basis of a water content of 82 percent. The distribution of radioactivity in glucose and glucose metabolites in the brain was determined by ion-exchange paper chromatography on protein-free filtrates (*7*). The concentration of brain glucose was measured by the glucose oxidase technique. Glucose and galactose concentrations in plasma were determined by gas-liquid chromatography. The glucose concentration was found to be 254 ± 10 and 292 ± 37 mg per 100 ml for control and galactose-fed chicks, respectively. Galactose was present only in the plasma of galactose-fed animals, 1010 ± 175 mg per 100 ml. The number of animals is indicated in parentheses.

Radioactivity of brain water (total counts per minute per milliliter)	Total brain glucose (μmole/g)	Percentage of radioactivity in brain	
		Glucose	Glucose metabolites
	Control animals		
186,790 (6)	1.31 ± 0.9 (6)	12.8 ± 4 (5)	87.1
	Galactose-fed chicks		
95,009 (6)	0.86 ± 0.14 (6)	12.9 ± 3 (6)	87.0

The apparent controversy regarding the biochemical bases for the neurotoxicity syndrome induced in chicks obscures the more important question of the validity of the galactose-toxicity syndrome in the chick as a model for the mental retardation observed in humans afflicted with galactose-1-phosphate uridyl transferase–deficiency galactosemia. Our findings of hyperosmolality coupled with reversibility of neurotoxicity within 24 hours of the cessation of galactose feeding strongly suggest that this is not a suitable analog. Hansen has stated that when galactose is removed from the chicks' diet, they develop normally (*6*). The finding that the syndrome is reversed when the blood glucose concentration is raised accentuates the acute nature of the syndrome in the chick in contrast to the subtle mental deficiency in the human. Human galactose toxicity is not associated with the same high concentrations of galactose that accompany the chick toxicity, nor is convulsive neurotoxicity part of the syndrome in humans. Hypoglycemia occasionally occurs in human uridyl transferase–deficiency galactosemia, but the postulate of a chronic lack of glucose in the brain as a cause of retardation has not been seriously considered since other human disorders, such as glycogen storage disease with a diminished availability of brain glucose, are not associated with mental retardation. Furthermore, patients with galactokinase-deficiency galactosemia have no mental retardation despite galactose ingestion and elevated blood galactose concentrations, two characteristics that these patients share with persons who are afflicted with uridyl transferase–deficiency galactosemia. Hence the etiology of the mental retardation must be related to biochemical disturbances apart from changes in the competition between galactose and glucose for entry into the brain. Thus, our own findings as well as those of Knull *et al.* have led us to discard this model and seek another that may better reflect the human galactose toxicity condition.

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References and Notes

1. H. J. Wells and S. Segal, *Fed. Eur. Biochem. Soc. Lett.* **5**, 121 (1969).
2. L. P. Kozak and W. W. Wells, *Arch. Biochem. Biophys.* **135**, 371 (1969).
3. ———, *J. Neurochem.* **18**, 2217 (1971).
4. S. Segal, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds. (McGraw-Hill, New York, 1972), p. 188.
5. J. H. Nordin, D. R. Wilken, R. K. Bretthauer, R. G. Hansen, H. M. Scott, *Poultry Sci.* **39**, 802 (1960).
6. R. G. Hansen, personal communication.
7. P. Cuatrecasas and S. Segal, *J. Biol. Chem.* **240**, 2382 (1965).
8. Supported by NIH grant AM-10894.

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