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Teratogenic Significance of Ionic and Fluid Imbalances

Abstract. *Some agents teratogenic to the chick embryo cause serum electrolyte and fluid imbalances which initiate an edema syndrome leading to malformations. Differences in ionic composition of serum and yolk of normal chicks help explain how these imbalances can be produced.*

A study of the effects of moderate hypoxia on young chick embryos has shown that many of the induced malformations can be attributed to a disturbance in fluid balance (*1*). This disturbance, which characterizes the edema syndrome, includes a marked increase in circulating and extracellular fluids, followed by the formation of clear blisters and hematomas which cause malformations mechanically. The edema syndrome can also be induced by Trypan blue (*2*) and injections of 0.01 to 0.02 ml of fresh egg albumen into the allantois of 4- to 5-day chick embryos. The latter can induce malformations in 20 percent (30 out of 149) of embryos treated at 4 days and 9 percent (9 out of 91) at 5 days (*3*). Less than 2 percent malformations were found in 225 controls that were injected with saline. All three agents can induce identical anomalies, indirectly caused by blisters, when applied to embryos of the same age. Because changes in the fluid volume of the embryo initiate the edema syndrome, we decided to study the composition of embryonic fluids in normal and experimental chicks.

We made assays of Na^+ and K^+ in normal extracellular and extra-embryonic fluids of the 5-day chick embryo (Table 1). These fluid compartments can be divided into two groups on the basis of the concentration of these electrolytes. Albumen and yolk, which are separated from the embryo by cellular membranes, are very high in K^+, and low in Na^+ and in total Na^+ and K^+. Blood serum, cerebrospinal, chorionic, and amniotic fluids are low in K^+, and high in Na^+ and in total Na^+ and K^+. The fluid of the allantois, which has an opening into the yolk sac, is intermediate. As might be expected from the data of Table 1, but contrary to the popular assumption that these fluids have equivalent osmotic pressures (*4*), we found that the depression of freezing point of albumen and subgerminal yolk were markedly lower than that of normal serum (*5*). These observations show that the osmotic relationships of the chick embryo to its surrounding fluids are more complex than generally believed.

Because of these differences between the internal and external fluids of the chick embryo we felt that fluid and ionic imbalances could be induced by a variety of physiological stimuli, so we exposed embryos to several teratogenic agents and tested the serum (Table 2). Saline-injected controls showed an increase in the concentration of potassium ions but no changes in the concentration of sodium ions. This change is probably the result of mild trauma from opening the egg. Occasional malformations are also found in these controls. Embryos subjected to hypoxia (*6*) or treated with albumen or Trypan blue, consistently showed a twofold increase in serum potassium and a significant decrease in serum sodium. The decrease in the concentration of sodium ions compared with that in normal sera, when the embryos were subjected to hypoxia or to treatment with albumen, was significant at the 0.1 percent level (*t*-test); when treated with Trypan blue, the decrease was significant at the 3 percent level. The lower total concentration of these two ions in experimental sera helps to interpret the edema which results from these treatments. The depression of freezing point of the serum of embryos exposed to hypoxia is substantially lower than that of normal serum (*5*).

The data clearly demonstrate that fluid and electrolyte imbalances are produced in the embryo by hypoxia, albumen, and Trypan blue. The edema syndrome is a common factor to all of them and leads to the development of similar malformations.

Although these data cannot be assumed to apply directly to the mammal, we feel a similar study would be a profitable approach to problems of mammalian teratology, because effects comparable to the edema syndrome have been described many times in mice and rats, both after the administration of Trypan blue (*2*) and vasopressin (*7*), and in deficiencies of linoleic and pantothenic acids (*8*), as well as in genetic mutants (*9; 10*).

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Table 1. Sodium and potassium in fluids of normal 5-day chick embryos. The results are expressed as milliequivalents per liter. Determinations were made on a Beckman DU spectrophotomter with a flam attachment, subsequent to perchloric acid digestion.

Fluid	No. of assays*	Na^+		K^+		Total Na^+ and K^+
		Av.	S.D.	Av.	S.D.	
Albumen	**7**	65	13.0	61.3	7.5	126
Subgerminal yolk†	**6**	98	1.6	19.4	3.72	117
Allantois	**7**	115	10.6	9.0	1.14	124
Blood serum	**15**	136	9.0	3.2	0.55	139
Cerebrospinal fluid	**7**	131	11.1	4.0	1.38	135
Chorion	**6**	133	5.5	4.1	0.79	137
Amnion	4	131	6.2	2.2	0.25	133

* Each sample obtained from different chicks except for serum and cerebrospinal fluid which were samples pooled from 2 to 3 embryos. † Clear yolk immediately underneath embryo.

Table 2. Serum sodium and potassium in 5-day chick embryos. The results are expressed as milliequivalents per liter. Experimental treatments: Hypoxia, 10.5 percent O_2 for 6 hours (*6*); fresh egg albumen, 0.02 ml injected into allantois; Trypan blue, 0.06 ml of a 0.1 percent solution injected into yolk sac; and NaCl, 0.02 ml of 0.85 percent saline injected into allantois. The serum samples from the embryos subjected to hypoxia were obtained within 1 hour after treatment was terminated. Other samples were obtained 5 to 6 hours after injection.

Treatment	No. of assays	Na^+		K^+		Total Na^+ and K^+
		Av.	S.D.	Av.	S.D.	
None	**15**	136	9.0	3.2	0.55	139
Hypoxia	**9**	117	10.7	6.5	1.06	124
Albumen	**7**	121	6.6	5.7	1.34	127
Trypan blue	**5**	122	11.6	6.6	1.14	129
NaCl	**5**	137	10.7	5.1	0.60	142

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Rhizomorph Production by Armillaria mellea Induced by Ethanol and Related Compounds

Abstract. Armillaria mellea *produced abundant rhizomorphs when grown on a chemically defined medium containing ethanol at concentrations as low as 50 parts per million. In the absence of ethanol no rhizomorphs were formed. Rhizomorph production was also stimulated by 1-propanol, isopropanol, and 1-butanol as well as by acetaldehyde (to a lesser extent). Potassium acetate had very slight stimulating effect, and methanol was completely ineffective.*

Armillaria mellea (Vahl) Quél. is a widely prevalent and important plant pathogen in California. This fungus is unique in its production of highly organized rhizomorphs whose development and morphology have been recently investigated by Townsend (*1*) and Snider (*2*). These structures function in the penetration of host roots by this pathogen. Investigations of the nutritional requirements for rhizomorph production and growth have been published (*2, 3*). In these studies it was necessary to include in the medium a complex substrate such as peptone or yeast extract in order to obtain vigorous development of rhizomorphs. During an investigation to determine the nutritional factors responsible for rhizomorph formation and growth it was found that ethanol would markedly stimulate rhizomorph development in a chemically defined synthetic medium.

The production of ethanol by fungi is well known (*4*). The utilization of ethanol as a sole carbon source for growth is less common but has been reported (*5, 6*). Miller and Halpen (*6*) reported that ethanol at certain concentrations supported abundant sporulation of yeast. However, the action of ethanol in stimulating growth or influencing the type of growth has received little attention. Springer and Gernet (*7*) reported that addition of 0.5 to 2 percent ethanol would stimulate the synthesis of citric acid by *Aspergillus niger*, and Cochrane (*8*) found that low concentrations of ethanol would stimulate germination of the macroconidia of *Fusarium solani*.

The basal medium in this investigation was composed of 5 g of glucose, 0.75 g of $MgSO_4 \cdot 7H_2O$, 1.75 g of KH_2PO_4, 2 g of L-asparagine, 1 mg of thiamine, and 20 g of agar in 1000 ml of distilled water. The *p*H of the medium was adjusted to 5.8. Early in the study the requirement of *Armillaria mellea* for thiamine and the poor growth on inorganic forms of nitrogen, reported by Garrett (*3*), were confirmed.

The fungus was grown in glass 4-oz (118 ml) prescription bottles containing 20 ml of medium. The bottles were laid flat to form a layer of agar measuring 90 by 45 by 5.0 mm. Inoculum consisted of 5-mm disks of water-agar containing mycelium of the fungus. The water agar was seeded with a small piece of rhizomorph and was ready for use in 2 to 3 weeks. Inoculum over 5 weeks of age was not used. After the test bottles had been seeded they were incubated in the dark at 25°C. The total length (in centimeters) of rhizomorphs formed was determined with a Keuffel and Esser map-reading device (*1, 2*). This method provided a satisfactory relative measurement of rhizomorph growth. Only qualitative evaluations of mycelial growth were made.

On the basal medium *A. mellea* showed good mycelial growth but did not produce rhizomorphs. When redistilled ethanol was added to give a final concentration of 150 or 1500 parts per million by weight (3.2 and 32 mmole/liter), mycelial growth was stimulated and rhizomorphs were produced (see cover). The first evidence of rhizomorph development was apparent after 5 to 6 days, and there was extensive development at 14 days. The stimulating effect of ethanol was detected at a concentration of 25 ppm. Optimum concentration appeared to be at 500 ppm, and very little increase in rhizomorph development occurred at 1000 and 2000 ppm ethanol. Distorted and stunted rhizomorphs resulted when the alcohol concentration was 4 percent. No inhibition occurred at 1 percent ethanol. The stimulatory effect of ethanol was observed with all of five isolates tested. The isolates all originated from single spores and varied in their ability to produce rhizomorphs.

When related compounds containing two carbon atoms were tested, acetate was only slightly stimulatory and acetaldehyde, while toxic at 0.01*M*, induced rhizomorph formation at lower concentrations (Table 1). Of other alcohols tested, methanol had no stimulatory activity whereas 1-propanol and 1-butanol markedly enhanced rhizomorph development (Table 1). Isopropanol was similar to 1-propanol in stimulatory activity. Compounds that stimulated rhizomorph development also stimulated mycelial growth. In all cases the *p*H of the medium after growth was 5.8 to 6.0.

When yeast extract (2 g/liter) or peptone (2 g/liter) were added to the basal medium, rhizomorphs were produced without the addition of alcohol. However, ethanol significantly increased rhizomorph production when it was added to medium containing either of these compounds. Rhizomorphs were produced on potato-dex-

Table 1. Effect of ethanol, related compounds containing two carbon atoms, and other alcohols, in different concentrations, on rhizomorph production by *Armillaria mellea*.

Conc. (mmole/liter)	Length (cm) at 14 days*					
	Ethanol	Acetaldehyde	Potassium acetate	Methanol	1-Propanol	1-Butanol
10.8	59.8 ± 2.8		17.5 ± 1.1	< 1.0	36.5 ± 1.3	79.3 ± 2.9
2.6	60.3 ± 4.3	21.3 ± 3.7	11.2 ± 6.0	< 1.0	54.5 ± 3.5	54.2 ± 4.7
1.08	28.0 ± 3.2	15.7 ± 3.5	2.5 ± 0.3		49.0 ± 4.4	43.7 ± 4.7
0.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

* Each value is the mean of at least six replications; standard error is indicated.