The Distribution of Injected ^{32}P in Transient Hyperammonemia in the Chick and Rat (35362)

KYE-WING CHOW, FREDERICK W. LENGEMANN, AND WILSON G. POND

Department of Animal Science, New York State College of Agriculture, Department of Physical Biology, New York State Veterinary College and Graduate School of Nutrition, Cornell University, Ithaca, New York 14850

Blood metabolite changes of hyperammonemic rats and pigs (1–3) indicate radical changes in energy metabolism afflicted animals. Intracerebral toxicity of ammonia was found to decrease basilar ATP and phosphocreatine levels in the rat (2). *In vitro* studies have shown that addition of ammonium salts inhibited oxygen uptake in phosphorylating rat liver mitochondria in media containing a phosphate-trapping system (glucose and hexokinase) (4, 5). Similarly, ammonium chloride added at concentrations between 2 and 15 m*M* was found to reduce ATP formation by phosphorylating rat and baby pig liver mitochrondria (6). At the same time, it was demonstrated that, at these levels, ammonium ions would complex stoichiometrically with phosphate and magnesium ions of the incubating media (pH 7.5). The complex formed was identified as magnesium ammonium phosphate, a salt insoluble at neutral and alkaline pH. The conditions under which complex formation took place *in vivo* were described 60 years ago by Law (7) and more recently by Rottschaefer *et al.* (8).

The purpose of the present study was to trace the fate of injected ^{32}P in the chick and rat made transiently hyperammonemic with low doses of ammonium carbonate just prior to ^{32}P administration.

Experimental Procedure. Rapidly growing, 4-week-old White Leghorn cockerels and Sprague–Dawley male rats, weighing 100 g, were used as the experimental subjects.

Ammonia doses were administered intraperitoneally in the form of 10% ammonium carbonate solution (pH 8.5) equivalent to 1.0 mmoles of ammonia/kg of body weight for the chick and 3.0 mmoles/kg of body weight for the rat.

Phosphoric acid ^{32}P (New England Nuclear, 2 mCi/ml) was diluted with distilled water and injected intraperitoneally immediately after administration of ammonia. Phosphorus-32 dose was approximately 10 μCi in 0.5 ml for the chick and 20 μCi in 0.5 ml for the rat.

Absorption periods were 50 min for the chick and 60 min and 20 hr (in metabolic cages), respectively, for the rat. Blood was collected in heparinized tubes from the jugular-carotid vessels of all animals for use in radioisotopic and chemical analyses. Tibias and livers were collected from both the chicks and rats; kidneys and 20-hr urine samples were also collected from the rats.

Blood analysis. Two ml of each blood sample were pipetted into 8 ml of 10% trichloroacetic acid. The protein-free filtrate from this was analyzed for ammonia, hexose, pentose, pyruvate, ketone bodies, urea, and inorganic phosphorus as described earlier (3), except that blood hexose was determined by the anthrone method (9). Two-ml aliquots of this filtrate were also diluted to 15 ml for radioactivity measurement as described below.

Tissue preparation. Livers and kidneys were removed immediately, rinsed, blotted dry, weighed, and dry-ashed overnight in porcelain crucibles at 500°. Tibias were similarly removed and ashed, but only their ash weights were taken. Ash from all tissues was dissolved in 2 ml of 5 *N* HCl, transferred into glass liquid scintillation counting vials, and diluted to 15-ml volume with distilled water.

Urine collection. Rats were kept in metabolism cages and urine samples were collected over a 20-hr period. The urine samples were diluted to 50 ml and a 15-ml aliquot was taken for 32P counting.

Measurement of radioactivity. The Cĕrenkov technique was employed (10); ^{32}P dosing standard was prepared by taking 0.1 ml of the dose solution and bringing it to a final volume of 100 ml with distilled water. One ml of the dosing standard was diluted to 15 ml in a counting vial and counted. Activities were expressed in percentage of dose per 100 g of wet weight for the livers and kidneys, per 100 ml for blood, and per gram of ash weight for tibias. Urine counts for the 20-hr collection were calculated on the basis of the injected ^{32}P as percentage of dose on the 20-hr collection.

Results. Chick study. Distribution of ^{32}P label. The incorporation of ^{32}P in the liver of the ammonia-intoxicated chicks 50 min after dosing was not different from that of the controls and would suggest comparable absorption in both cases (Table I). The accumulation of the label into the tibia, however, was almost twice as great for the control as for the intoxicated birds ($p<.001$). The label appearing in the blood as inorganic phosphate was about 30% lower in the ammonia intoxicated animals as for the controls and was significant ($p<.001$).

Changes in blood metabolities. The pattern of changes in blood metabolites in the transiently hyperammonemic chick (Table II)

TABLE I. Distribution of Injected ^{32}P in the Hyperammonemic Chick.

Tissue	Treatment[a]	
	Control	Ammonia
Liver (% dose/100 g of wet wt)	5.41 ± 0.58	5.34 ± 0.27
Tibia (% dose/g of ash wt)	0.63 ± 0.03	0.37 ± 0.03[b]
Blood (P_i) (% dose/100 ml)	10.37 ± 0.69	6.87 ± 0.47[b]

[a] Values represent mean ± SE of 9 birds at 50 min after ammonia administration.
[b] $p<.001$.

TABLE II. Blood Metabolites in the Hyperammonemic Chick.

Metabolite (mg/100 ml)	Treatment[a]	
	Control	Ammonia
Ammonia	0.23 ± 0.07	0.34 ± 0.02[b]
Hexose	127.0 ± 5.3	183.4 ± 9.1[c]
Pyruvate	0.29 ± 0.01	0.33 ± 0.01[b]
Ketones	0.50 ± 0.03	0.58 ± 0.01[b]
Pentose	21.4 ± 0.5	24.8 ± 0.05[c]
Inorganic phosphate	13.6 ± 2.1	11.5 ± 1.3

[a] Values representing mean ± SE of 9 birds at 50 min after ammonia administration.
[b] $p<.05$.
[c] $p<.001$.

resembles that reported for the acutely hyperammonemic pig (3). Blood ammonia was still elevated 50 min after ammonia administration. Blood hexose, pentose, pyruvate, and ketone levels were increased 40 ($p<.001$), 16 ($p<.001$), 14 ($p<.05$) and 16% ($p<.05$), respectively. Blood inorganic phosphorus decreased 15%; this decrease was not significant.

Rat study. Distribution of ^{32}P label. The incorporation of injected ^{32}P into the tibia of the ammonia-intoxicated rat, for the 1-hr period, was reduced 30% ($p<.005$) compared to the control. There was no difference when the experimental period was 20 hr (Table III). In the 1-hr study, the levels of ^{32}P in both liver and kidney, but not blood, were significantly higher ($p<.005$) in the ammonia-treated animal. Differences were not significant at 20 hr. Analyses of variance for the 2×2 factorial experimental design performed on liver, kidney, and tibia data showed significantly increased incorporation of label with time for all three tissues (Table IV).

Excretion of ^{32}P label. Urine collected in the 20-hr study showed that the ammonia-treated animals excreted 40% more ($p<.001$) label than the control (Table III).

Changes in blood metabolites. In the 1-hr study, the inorganic phosphorus in the treated animal was significantly lower ($p<.001$) than for the control (Table V). The elevated blood urea in the treated animal ($p<.001$) provides evidence of a transi-

TABLE III. The Influence of Ammonia and Absorption Time on the Distribution of Injected ^{32}P in the Rat.

Tissue	Treatment			
	1 hr[a]		20 hr[b]	
	Control	Ammonia	Control	Ammonia
Liver (% dose/100 g of wet wt[c])	8.39 ± 0.20	11.17 ± 0.55[e]	21.39 ± 0.74	23.15 ± 0.94
Kidney (% dose/100 g of wet wt[c])	38.19 ± 1.37	43.62 ± 1.66[e]	141.10 ± 3.60	150.80 ± 3.80
Tibia (% dose/g of ash wt[c])	2.17 ± 0.09	1.52 ± 0.14[e]	2.63 ± 0.12	2.39 ± 0.09
Blood (P_i) (% dose/100 ml[d])	7.06 ± 0.09	6.76 ± 0.26	—	—
Urine (% dose[d])	—	—	1.22 ± 0.13	1.70 ± 0.15[e]

[a] Values representing mean ± SE of 10 animals; [b] 8 animals.
[c] Statistics: 2 × 2 factorial analysis of variance.
[d] Statistics: Student's *t* test.
[e] $p < .001$.

ent hyperammonemic state. Blood metabolites in the 20-hr study showed no differences between treated and control animals.

Discussion. The data of these studies indicate that ammonia toxicity in the chick and in the rat may interfere with phosphate metabolism while not significantly affecting absorption from the peritoneal cavity. In both the chick and the rat, at about 1 hr after dosing, there was reduced accumulation of ^{32}P into the tibia. In the rat, but not in the chick, there was concurrent accumulation of label in the liver. In the rat also, there was accumulation of label in the kidney following ammonia toxicity.

The apparent immobilization of phosphate by ammonia as judged by tibia ^{32}P could have occurred in the same manner observed in both *in vitro* and *in vivo* studies (6–8), *i.e.*, through formation of magnesium ammonium phosphate. If this assumption is correct, the distribution of the injected ^{32}P in the ammonia-intoxicated rat may be explained as follows: the complex, present in a form that could not be incorporated into bone tissue, was treated by the animal body as a waste product and subjected to detoxification and excretion. The increased levels of label in the liver and kidney, together with increased excretion of label into urine, lend support to this reasoning. That the liver, or some other organ, had some capacity to detoxify this complex was evidenced by the lack of a difference in the ^{32}P content of tibia at 20 hr. This capacity might have been inherent in the liver or tied to the incipient metabolic acidosis produced in hyperammonemia (the complex is soluble at acid pH).

TABLE IV. Auxiliary Treatment Sums of Squares for ^{32}P Incorporation into Rat Liver, Kidney, and Tibia.

Treatment comparison	*df*	Liver[a]		Kidney[a]		Tibia	
		Mean square	*F*	Mean square	*F*	Mean square	*F*
Between times within control	1	0.3172	138[c]	0.0424	25[c]	0.142	1.43
Between times within NH_3 treated	1	0.1324	58[c]	0.0161	9.5[c]	3.010	30[c]
Between treatments within 1 hr	1	0.0661	29[c]	0.0148	8.7[b]	3.184	32[c]
Between treatments within 20 hr	1	0.0045	1.9	0.0029	1.7	0.180	1.8

[a] Logarithmic transformation was performed on raw data prior to analysis of variance.
[b] $p < .01$.
[c] $p < .005$.

TABLE V. The Influence of Ammonia and Absorption Time on Blood Metabolites in the Rat.

	Treatment			
	1 hr[a]		20 hr[b]	
Metabolite (mg/100 ml)	Control	Ammonia	Control	Ammonia
Ammonia	0.09 ± 0.0	0.10 ± 0.0	0.10 ± 0.0	0.12 ± 0.01
Hexose	112.9 ± 3.5	125.5 ± 6.1	90.0 ± 3.1	81.0 ± 4.3
Pyruvate	0.18 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.23 ± 0.02
Pentose	26.9 ± 0.4	27.5 ± 0.8	29.9 ± 0.4	29.8 ± 0.5
Ketones	0.25 ± 0.02	0.31 ± 0.03	—	—
Inorganic phosphate	15.1 ± 0.2	13.7 ± 0.2[c]	—	—
Urea	27.4 ± 0.7	39.8 ± 2.1[c]	—	—

[a] Values representing mean ± SE of 10 animals; [b] 8 animals.
[c] $p < .001$.

Detoxification must be a slow process since blood urea continued to be high even though blood ammonia had returned to normal after 1 hr. The kidney, on the other hand, probably dealt with this complex by slow excretion into the urine (7), although concurrent detoxification with or without conservation of Mg and/or P, cannot be ruled out.

Given the reactivity of ammonia with phosphate and magnesium ions, and the extreme insolubility of the formed complex (K_s^0: 2.5×10^{-13}), peripheral blood ammonia levels encountered in acute ammonia toxicity (2 mg/100 ml, or more) would result in the complexing of almost the entire pool of inorganic phosphate and more than 50% of magnesium ions from the systemic blood. Acute ammonia toxicity, then, will necessarily differ from mild toxicity in that, both magnesium and phosphate ions will be depleted to dangerously low levels. Indeed, the physical manifestiations of ammonia toxicity in its acute from bear strong resemblance to hypomagnesemia in animals; and the cause of grass tetany (a fatal disease of cattle usually associated with hypomagnesemia) described by Sjollema (11) has often been ascribed to ammonia toxicity contracted through foraging by the ruminant on pastures freshly fertilized with high amounts of ammonium salts (12). Hypomagnesemia has also been described to lead to hypophosphatemia (13). At mildly toxic levels, such as used in this study, probably only phosphate depletion would be of concern.

The present data demonstrate that the chick is susceptible to even low doses of ammonia. In acute toxemia in the chick, it has been observed that death was almost immediate with fleeting convulsions, unlike in the rat, when protracted coma with accompanying sporadic convulsion is almost always observed. This difference in response to ammonia may be due to the absence of a urea cycle in the chick. The pattern of changes in organic blood metabolities in the hyperammonemic chick in this study resembles those reported on the acutely ammonia intoxicated rat and pig (1–3), suggesting the existence of a primary event in ammonia toxicity which probably is not species specific.

It is not the intent to explain all the changes in organic blood metabolites observed during ammonia toxicity. There is reason, however, to believe that any sudden depression of phosphate ions, and possibly of magnesium ions as well, at the tissue level would have a broad effect on energy metabolism of the animal as evidenced by drastic changes in levels of hexose, pentose, pyruvate, and ketone bodies in the peripheral blood of ammonia-intoxicated animals. The results of this study represent the first direct evidence that low doses of ammonia administered to laboratory animals adversely affected inorganic phosphate metabolism.

Summary. The distribution of injected ^{32}P was studied in the mildly hyperammonemic chick and rat. Ammonia toxicity in the chick resulted in decreased accumulation of ^{32}P in

the tibia ($p<.001$) and decreased level of labeling in the inorganic phosphate fraction of blood ($p<.001$). Toxicity in the rat also resulted in decreased accumulation of label in the tibia ($p<.005$), but increased incorporation in the liver ($p<.005$), kidney ($p<.005$), and urine ($p<.001$), indicating that injected ammonia probably converted inorganic phosphate into a form that was eventually treated by the body as a waste product. Changes in organic blood metabolites accompanying ammonia toxicity in the chick paralleled those described earlier for the rat and pig.

The results of this study represent the first direct evidence that low doses of ammonia administered to laboratory animals interfere with phospate metabolism.

The authors thank Dr. Malden C. Nesheim, Department of Poultry Science, Cornell University, for the supply of the chickens and Miss Ruth E. Whetzel for secretarial assistance.

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Received Sept. 2, 1970. P.S.E.B.M., 1971, Vol. 136.