Many phases of the biology of the *Coturnix* have not been investigated. The bird's response to various types of light stimuli needs to be studied. Hormonal studies conducted on the *Coturnix* would be of great interest. At present, research is being conducted in our laboratory on the normal embryology and the gonadal development of these birds.

A study of anomalies occurring in the embryos and of the genetic and environmental factors affecting them would be of interest. We have found a number of cases of incomplete twinning. These twins, with one head and two bodies, have been found to be alive in the eggs as late as the 13th day of incubation. Several cases of perocephaly, such as have been recorded for the chick by Landauer (*5*), have been found, including cases where the cranium completely failed to form over the cerebrum.

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Hypoxanthine in Rosy and Maroon-like Mutants of Drosophila melanogaster

Hadorn and Schwink (*1, 2*), in an examination of the pleiotropic effects of the rosy^2 (*ry*^{2}; location, 3: 51 ±) mutant of *Drosophila melanogaster,* demonstrated that the mutant is deficient in isoxanthopterin and contains an excessive quantity of 2-amino-4-hydroxypteridine. At the same time, Forrest, Glassman, and Mitchell (*3*) observed that the maroon-like (*ma-l*; location, 1: near *beadex*) mutant of *Drosophila melanogaster* is deficient in an enzyme capable of catalyzing the oxidation of 2-amino-4-hydroxypteridine to isoxanthopterin.

Subsequent work has provided quantitative data on pteridine concentrations in the *ry* mutants (*4*), evidence that both the *ry* and *ma-l* mutants are deficient in the pteridine-oxidizing enzyme (*5*), and evidence that the enzyme involved is xanthine dehydrogenase (*5*). As indicated elsewhere (*5, 6*), this enzyme deficiency should lead to an accumulation of hypoxanthine as well as 2-amino-4-hydroxypteridine. This expectation has been found to be correct by observations of chromatograms from the mutants and wild-type *Drosophila* and by the isolation and identification of hypoxanthine from extracts of *ry*^{2} adult flies. It has also been observed that hypoxanthine rather than uric acid is a major nitrogenous excretion product of the mutant flies.

Table 1. Absorbing compounds in mutants and wild-type *Drosophila melanogaster.* The presence or absence of substances corresponding to hypoxanthine and uric acid was consistent in all five solvents. Approximately 5 percent of maximum quantities would have been detected by the methods used.

Extract	Uric acid position	Hypoxanthine position
Wild (Oregon)	+	−
ry^1	−	+
ry^2	−	+
ma-1	−	+

Chromatograms of squashed animals (*7*) or boiled extracts, and of known purines as controls, were developed in the following solvents: (i) *n*-propanol (2 parts): 1 percent aqueous NH_3 (1 part); (ii) isopropanol (3 parts): 1*M* formic acid (1 part); (iii) *n*-propanol (3 parts): 2*N* HCl (1 part); (iv) isopropanol (7 parts): 1 percent aqueous NH_3 (3 parts); (v) 5 percent (by volume) acetic acid in water. Positions of all absorbing materials on the chromatograms were noted with the aid of a Mineral Light ultraviolet lamp (principal emission, 2537 A). Results pertinent to this discussion are summarized in Table 1. By extraction of absorbing materials, followed by spectrophotometric measurements, it was determined that the mutants that accumulate hypoxanthine contain from 1 to 3 μg of the purine per animal (approximately 1 mg wet weight).

Chromatograms of excretion products scraped from the walls of culture bottles yielded a picture similar to that indicated in Table 1.

For the isolation of hypoxanthine, 50 g of washed adult *ry*^{2} flies were extracted with 350 ml of boiling water. After filtration and addition of a concentrated solution of lead acetate (3 g), the solution was adjusted to *p*H 9 with NH_4OH. After centrifugation, the precipitate was discarded and the supernatant was adjusted to *p*H 10. The resulting precipitate was removed by centrifugation, resuspended in water and treated with H_2S. The supernatant solution, after removal of lead sulfide, was evaporated to dryness and then extracted with 2 ml of hot water. This extract was streaked on a sheet of Whatman No. 4 paper and chromatogramed in solvent i (Table 1). Absorbing material (2537 A) at the hypoxanthine position was eluted with water, and chromatography was repeated with solvent v (Table 1). The eluate from the second chromatogram was evaporated to dryness, and the product was dissolved in 1 ml of hot water. Following treatment with a small amount of charcoal, the purine was crystallized from hot water with addition of ethanol to a slight turbidity. The yield after two recrystallizations was 5.5 mg. The product was chromatographically identical with hypoxanthine in the five solvents given above. It was also identical with hypoxanthine with respect to absorption spectra in 0.1*N* NCl ($\lambda_{max.} = 248$ mμ; log $E_{max.} = 4.03$) and in 0.1*N* NaOH ($\lambda_{max.} = 261$ mμ; log $E_{max.} = 4.06$).

Although hypoxanthine has been isolated in a pure form only from *ry*^{2} mutant, chromatographic and spectral characteristics of corresponding materials from the other mutants are in excellent agreement with the conclusion that the mutants tested accumulate and excrete hypoxanthine in the place of uric acid. It should also be noted that *ry*^{1} and *ry*^{2} may be genetic duplicates but that *ma-1* represents quite a different genetic locus. Nevertheless, the morphological and biochemical phenotypes are surprisingly similar. It is also of interest to note that in *Drosophila* the degradation of purines and their excretion as uric acid are not essential, nor is hypoxanthine particularly toxic to the animal even in the large quantities accumulated. However, at higher temperatures the *ry*^{2} mutant behaves as a late pupal or early imaginal semilethal (*1*), and it is possible that the purine accumulation is a contributory factor to this phenomenon (*8*).

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Partial Pressure of Ammonia in Alveolar Air

Abstract. The partial pressure of ammonia in alveolar air was measured and found to be the same (within the limits of experimental error) as the calculated partial pressure of ammonia in arterial plasma. It is likely that ammonia is equilibrated between alveolar air and the blood during its passage through the pulmonary capillaries.

The feeding of ammonium salts to cirrhotics or to dogs and human beings with portocaval shunts induces symptoms which are similar to those of hepatic coma (*1*). Ever since the work of Hahn *et al.* (*2*), evidence has been presented at times linking elevated blood ammonia levels and hepatic coma. However, Conway's (*3*) extensive studies and his conclusion that there is no ammonia in normal blood have led to controversy over the interpretation of measurements of blood ammonia. Recently we have suggested (*4*) that it might be the free rather than the total blood ammonia which is of significance in the clinical manifestations of hepatic coma, and that therefore we might find a better correlation with the partial pressure of ammonia (P_{NH_3}). Measurement of alveolar P_{NH_3} and comparison of it with the P_{NH_3} calculated from the plasma *p*H and total ammonia would help in the resolution of this problem. Poppell (*5*) has demonstrated the presence of ammonia in the expired air of dogs with Eck fistulas and of normal dogs. This encouraged us to attempt more quantitative measurements (*6*).

Mongrel dogs with portocaval shunts were deeply anesthetized with Nembutal (*7*). Because of the possibility of ammonia formation by bacterial action in the mouth, tracheal intubation was used. A double glass cannula with inflatable balloon was inserted into the trachea to within a few inches of the carina. The dog was ventilated via one side of the cannula with H_2SO_4-washed air by means of a variable speed respirator. End-expiratory air, sampled through the other half of the cannula by means of a variable speed pump (*8*), was passed through two ammonia absorbers in series and collected into a Douglas bag. The sampling was controlled by a valve in the cannula which was synchronized with the respirator to open at the end of expiration and close before the start of inspiration. During the gas-sampling period, two samples of arterial blood were taken anaerobically into heparinized syringes. Temperature was recorded by an oesophageal telethermometer. In test runs in which a 5-gallon gas reservoir was used in place of the dog, no ammonia was ever picked up in the ammonia absorbers.

The *p*H of the blood was measured with a Cambridge model R *p*H meter with a water-jacketed glass electrode. Plasma CO_2 was determined by the Van Slyke (*9*) manometric method, and the P_{CO_2} was calculated from the factors given by Milch *et al.* (*10*). The CO_2 in the gas sample was measured by the Scholander micromethod (*11*). The total plasma ammonia was measured by a modification of the method of Seligson and Hirahara (*12*). The P_{NH_3} of plasma was calculated, using the solubility coefficients for human plasma (*13*). The ammonia in the expired air was collected in a column of glass beads moistened with .02*N* H_2SO_4, in a 250-ml cylindrical separatory funnel. The ammonia was then released with saturated K_2CO_3, diffused to a drop of acid on a glass rod in the stopper, and determined with Nessler's reagent.

The results are summarized in Table 1. The last two columns show, respectively, the measured alveolar P_{NH_3} and the arterial P_{NH_3} calculated from the plasma total ammonia, the *p*H, and the solubility coefficient of ammonia (*13*). The correspondence between the two is very good, considering the cumulative errors in the experiments and particularly considering the fact that the alveolar P_{NH_3} is an average over a fairly long collection period whereas the calculated plasma P_{NH_3}'s are for two points in the collection period.

Recently we have measured the ammonia in the alveolar air of two normal dogs and have again obtained adequate checks between the measured alveolar P_{NH_3} and the calculated arterial P_{NH_3}. Although equilibration periods of at least 1 hour were used prior to the collection periods, arterial blood *p*H's varied by ± .02 *p*H units, and occasionally by more, and the plasma total ammonia often changed somewhat. The check between the alveolar P_{NH_3} and the calculated plasma P_{NH_3} is independent corroborative evidence that the total plasma ammonia measured by our method (*12*) is of the correct order of magnitude and corresponds to the amount present in plasma in vivo. Our measurements of normal human blood ammonia have checked with those of Calkins (*14*), who found, by means of Conway's method, a mean value of 0.8 μg of NH_3 nitrogen per milliliter of normal human blood. It would appear that the statement of Conway that there is less than 0.1 μg of NH_3 nitrogen per milliliter of normal blood may be incorrect. The difference in results may be accounted for by variations in the technique of handling the blood samples, since Conway's method is sound.

Our measurements establish that (i) ammonia is present in alveolar air and (ii) the amount present is of the order

Table 1. Alveolar P_{NH_3} experiments. Each experiment occurs as a double entry corresponding to the two arterial blood samples. In each experiment there was one gas collection. The two arterial samples were taken during the gas collection period.

Date	Dog No.	Temp. of dog (°C)	Gas			Arterial			Alveolar air NH_3 (10^{-4} meq)*	Arterial plasma NH_3 (meq/lit.)	Alveolar air P_{NH_3} (10^{-4} mm-Hg)	Calcd. arterial P_{NH_3} (10^{-4} mm-Hg)
			Collection time (min)	Vol. (lit. STPD)	P_{CO_2} (mm-Hg)	*p*H	CO_2 (meq/lit.)	P_{CO_2} (mm-Hg)				
2/5	x874	37	202	101	34.8	7.41	26.7	41.3	5.4	0.178	1.0	1.0
2/5	x874	36.9	202	101	34.8	7.43	25.8	38.2	5.4	0.112	1.0	0.7
2/10	B45	37.3	135	72.9	29.4	7.41		35.9	3.9	0.26	1.0	1.5
2/10	B45	37.3	135	72.9	29.4	blood sample lost			3.9		1.0	
2/12	x874	37.0	120	68.4		7.41			4.6	0.212	1.1	1.2
2/12	x874	36.8	120	68.4		7.40			4.6	0.213	1.1	1.2
2/17	x874	37.0	149	65	30.5	7.38	18.0	29.8	3.0	0.131	0.7	0.7
2/17	x874	37.2	149	65	30.5	7.43	21.1	31.3	3.0	0.123	0.7	0.8
2/19	x1100†	39.0	80	50.7	18.5	7.54	16.6	19.3	2.0	0.097	0.6	0.9
2/19	x1100†	38.8	80	50.7	18.5	7.53	15.6	18.6	2.0	0.099	0.6	0.9
2/24	x1100	37.0	110	57.4	42.3	7.38	27.7	45.9	1.5	0.119	0.4	0.6
2/24	x1100	37.3	110	57.4	42.3	7.39	25.8	41.8	1.5	0.132	0.4	0.7
2/26	x916‡	36	110	81		6.99	28.4	107.6	2.4	0.40	0.5	0.8
2/26	x916‡	36.2	110	81		6.99	27.3	103.4	2.4	0.185	0.5	0.4
3/3	x1100‡	37.8	100	65.4		7.00	32.5	123.1	1.6	0.150	0.4	0.4
3/3	x1100‡	37.6	100	65.4		7.02	31.9	118.6	1.6	0.188	0.4	0.5

* The volume of alveolar air collected was calculated as the volume of gas collected multiplied by the ratio of the gas P_{CO_2} to the arterial P_{CO_2}. In the three experiments in which gas P_{CO_2} was not measured, the volume of gas collected was used for the alveolar air collected.
† Hyperventilated.
‡ Ventilated with a mixture of 10 percent CO_2 and 90 percent O_2.