an NH_2-terminal glutamic acid (called glutamyl peptide), a dipeptide identified as Ala-Arg, and free arginine. Since trypsin cleaved the Lys-Ala (B_{29}-B_{30}) bond to give desalanine insulin and since the expected molar ratios of alanine were accounted for in the glutamyl peptide, it followed that the peptide Ala-Arg represented positions B_{30}-B_{31} (*21*). Two arginine residues were found in a chymotryptic fragment (B_{27}-B_{54}) isolated from a digest of intact proinsulin by a combination of G-50F Sephadex and Dowex 50-X4 chromatography; hence, the position of the free arginine was established as B_{32}. The amino acid sequence (Fig. 2) of the 31-residue glutamyl peptide (B_{33}–B_{63}) was determined by the combined Edman degradation-dansylation procedure with chymotryptic peptides isolated from a Dowex 50-X4 column eluted with pyridine-acetate buffers. Chymotryptic digests yielded peptides B_{33}–B_{54}, B_{55}–B_{63}, B_{57}–B_{63}, B_{33}–B_{44}, B_{45}–B_{51}, B_{45}–B_{48}, B_{49}–B_{51}, B_{52}–B_{54}, and B_{55}–B_{56}. Carboxypeptidase A digests were used to confirm the Glu-Leu (B_{43}–B_{44}) and the Leu-Gln-Ala-Leu (B_{51}–B_{54}) sequences. Treatment of the glutamyl peptide with trypsin and carboxypeptidase B established that the COOH-terminal dipeptide was Lys-Arg (B_{62}–B_{63}). Thus, the terminal connection between the fragment and the A chain was deduced to be Arg-Gly (B_{63}–A_1). The complete amino acid sequence of the connecting peptide (B_{31}–B_{63}) is shown in Fig. 2 as it is incorporated into the proposed primary structure of porcine proinsulin.

The physiologic significance of proinsulin and the mechanism responsible for the transformation of proinsulin to insulin are not known. Our study shows that trypsin, if involved at all, cannot be the sole releasing enzyme since trypsin digestion in vitro leads to the formation of desalanine insulin, not insulin. This finding was to be expected in view of the known specificity requirements for trypsin. Possibly trypsin or a trypsin-like enzyme is involved in the cleavage of the B_{63}–A_1 bond, whereas another enzyme (or enzymes) may hydrolyze the basic residues around the terminal B_{30}–B_{31} connection to give insulin. In support of this possibility, we have in fact, found that the B_{63}–A_1 bond (Arg-Gly) is hydrolyzed first. This was observed during carefully controlled tryptic digestions in which a significant amount of NH_2-terminal glycine was liberated.

Our data along with the work of Steiner and co-workers provide strong evidence that insulin is synthesized as a single-chain precursor which is subsequently converted in a zymogen-like manner as hypothesized (*22*). However, the exact nature and location of such a transformation and the physiologic importance of this precursor concept remain to be determined.

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Abnormal Water Balance in a Mutant Strain of Chickens

Abstract. *Polydipsia and polyuria are pronounced in chickens of a selected strain and this diabetes insipidus is inherited. The kidneys of such birds are capable of an antidiuretic response when lysine vasopressin or arginine vasotocin is injected. Osmotic pressure and sodium concentration of the plasmas of normal and mutant chickens are identical. Chicks predicted to have diabetes insipidus on the basis of parental pedigree are polydipsic.*

Excessive drinking (polydipsia) and urinary output (polyuria) are present in many commercial chicken flocks. These conditions have been recognized among birds placed in individual cages for various biological studies and for commercial egg production. Watery droppings were discovered in a strain of chickens maintained at Pennsylvania State University in 1957 (*1*). During 5 years this condition was associated with polydipsia and was characteristic of a few pedigreed female birds in only one of two strains which were hatched and reared in the same way. Results of extensive breeding experiments started in 1963 support the idea that polydipsia and polyuria in this strain of chickens are inherited (*2*). The condition can thus be characterized as hereditary diabetes insipidus (DI). For experimental purposes, two inbred strains of white Leghorn chickens were developed, one normal and the other possessing DI. As judged from the amount of water drunk or from the ratio of the amount of water drunk to the amount of food eaten, inbreeding of the DI line since 1963 has resulted in no increase in the severity of the disease. Similar measures of the normal line have also remained fairly constant. Progeny (F_1) of reciprocal crosses of the two lines in 1966 were normal. Segregation of normal and DI traits

Table 1. Water intake and excretion in normal and DI birds. Numbers in parentheses are means and standard deviations of values above.

Body weight (g)	Drinking (% weight / day)	Urine (% weight / day)	Na (mmole/liter)	Osmotic pressure (milliosmolal)	Na loss (mmole/day)
		Male DI chickens			
2628	41.6	18.3	12.6	85	6.3
2580	40.0	16.8	13.5	95	5.4
2400	26.0	5.5	15.3	155	1.87
2240	25.6	13.1	9.5	89	2.46
2400	23.2	4.54	22.9	133	2.4
2624	20.4	8.08	14.2	94	2.98
3480	18.5	7.10	14.1	104	3.25
2920	16.3	5.27	17.0	166	2.47
2604	16.3	3.85	23.6	128	2.34
	(25.3 ± 9.1)	(9.16 ± 5.18)	(15.9 ± 4.4)	(116.5 ± 28.3)	(3.27 ± 1.44)
		Male normal chickens			
2155	4.8	< 0.14			
2161	6.55	< 0.45			
2338	9.55	< 0.70			
2375	7.9	< 1.2			
	(7.20 ± 1.75)				
		Female DI turkeys			
5825	28.9	18.9	4.1	40	4.6
8022	12.0	4.7	23.4	196	7.9
8130	11.2	4.1	24.9	186	7.3

among progeny of sibling matings of the F_1 was approximately 3:1. No sex linkage was indicated. Back crosses remain to be carried out. Some difficulties are encountered in separating the phenotypes, but careful attention to food consumption, body weight, sex, and particularly the record of egg laying has resolved much of the problem. Chickens with diabetes insipidus appear to be normal in viability, fertility, food consumption, and body weight; the number, weight, albumen quality, and shell thickness of the eggs produced are normal (*2*). The best way to recognize the trait is by measurement of water consumption and excretion.

In Brattleboro rats with hereditary hypothalamic DI, DI is due to an autosomal recessive gene, probably causing decreased or blocked synthesis of antidiuretic hormone (ADH) in the hypothalamus (*3*). These rats excrete an average of 70 percent of their body weight per day as urine. Their serum osmolality is elevated, and the gene appears to be associated with various degrees of inviability and sterility. Prolonged treatment with ADH corrects the polydipsia, polyuria, and the elevated serum concentration.

We have studied physiological features of hereditary polydipsia and polyuria in the mutant chickens. This is necessary before an attempt can be made to explain the altered mechanism of genetic control in the DI birds. Comparison of birds with the Brattleboro rats promises to be interesting since water metabolism is somewhat different in the two species. In birds, reabsorption of water from the urine may take place in the large intestine or cloaca (*4*), sites other than the kidney at which the chicken antidiuretic hormone (arginine vasotocin, AVT) might act (*5*).

We used chickens raised on the Pennsylvania State University Poultry Farm. They were fed commercial rations appropriate for their age and housed in individual cages, except when crowded conditions required placement in pens. Changes in surroundings usually disturbed drinking and feeding rates, so time was always allowed for birds to resume normal eating and drinking. In a typical drinking experiment, the chicken would be placed in an individual metabolism cage, and given free access to water and food. After several days when the bird had become accustomed to the cage, measurements of food intake, water consumption, and urinary water production began. Urine is here defined as the liquid portion of the excrement, collected in a container under the funnel floor of the metabolism cage. However, these fluids are probably different in composition from fluid collected from the ureters (*4*). Most of the fecal solids were left on the collecting pan or the screen covering the entrance to the urine jar. Body weights were taken at the beginning and the end of the experimental period and averaged. If a substantial decrease in weight or food consumption occurred, the data for the entire experiment were discarded. Blood samples were taken by heart puncture; calcium heparin was used to prevent clotting. Plasma sodium concentration was determined on a Coleman flame photometer (model 21). Plasma osmotic pressure was measured on a Mechrolab vapor pressure osmometer (model 301A).

The mean drinking response in the DI group was more than three times that of the normal (Table 1). Cloacal urine production was correspondingly high. Most or all of the "urine" collected from the inbred normal birds was water that had dropped from the beak. The difference in cloacal water loss between normal and DI birds was large. The feces of DI birds were very loose and watery. Three female turkeys which apparently have DI were also studied (Table 1). The condition is

Table 2. The effect of age on excessive water intake in the chicken. Values for body weight and drinking are means ± standard deviation with range in parentheses.

Year tested	Number	Age (weeks)	Predicted phenotype	Body weight (g)	Drinking (% weight / day)
1966	17	3	DI	214 ± 36 (137 – 258)	44.3 ± 16.3 (25.8 – 71.3)
1966	14	3	Normal	183 ± 18 (157 – 215)	22.8 ± 6.1 (17.3 – 41.5)
1966	23	4	DI	331 ± 40 (236 – 412)	32.2 ± 7.3 (21.9 – 47.5)
1966	8	4	Normal	320 ± 16 (295 – 346)	14.5 ± 1.5 (11.8 – 15.9)
1967	8	4	DI	254 ± 23 (215 – 285)	43.9 ± 6.3 (36.2 – 55.4)
1967	15	4	Normal	200 ± 27 (148 – 229)	21.4 ± 3.7 (17.0 – 25.0)
1967	8	12–28	DI	1654 ± 85 (1550 – 1780)	28.7 ± 8.9 (13.1 – 42.7)
1967	15	12–28	Normal	1532 ± 307 (1060 – 2100)	8.5 ± 1.5 (5.7 – 10.9)

fairly similar; one turkey had urine of lower osmotic pressure than any of the chickens. Mean urine osmotic pressure of homozygous male DI rats is 126 milliosmolal (*6*), which is only slightly higher than the value for DI chickens. In DI chickens there is an inverse, curvilinear relation between the rate of urine flow and the osmotic pressure. A similar function is suggested for sodium concentration.

The amount of water drunk can be expressed as the percentage of body weight per day or as the ratio of the amount of water drunk to the amount of food eaten. An adult male DI bird will drink more than 12 percent of its body weight per day and have a ratio of water drunk to food eaten greater than 2 (*2*). The figures for normal birds will be correspondingly less. These two estimates of the degree of DI can be used interchangeably.

Twelve normal adult male chickens drinking an average of 5.5 ± 0.5 percent of body weight per day (ratio of water to food, 1.1 ± 0.1) had a mean hematocrit of 39.4 ± 2.7 percent, a mean plasma sodium concentration of 147.5 ± 3.4 mmole/liter, and a mean plasma osmotic pressure of 318.7 ± 6.5 milliosmolal. Eight adult male DI birds drank an average of 23.9 ± 6.4 percent of body weight per day (ratio of water to food, 4.5 ± 0.1), and had a mean hematocrit of 38.8 ± 1.8 percent, a mean plasma sodium concentration of 146.5 ± 3.6 mmole/liter, and a mean plasma osmotic pressure of 315.5 ± 6.6 milliosmolal. Four normal adult female chickens drank on the average 11.8 ± 1.6 percent of body weight per day (ratio of water to food, 2.1 ± 0.3) and had a mean plasma sodium concentration of 146.5 ± 4.8 mmole/liter. Sixteen adult female DI birds had a mean water consumption of 40.8 ± 13.5 percent of body weight per day (ratio of water to food, 7.5 ± 3.1) and a mean plasma sodium concentration of 145.3 ± 6.0 mmole/liter. Thus the hematocrit, plasma sodium concentration, and plasma osmotic pressure are the same in normal and DI birds, indicating that the DI birds are able to maintain normal plasma sodium balance. Ample sodium is available in the food (about 10 mmole/day) to counteract the large fecal loss which occurs. Total amounts of sodium lost in feces of DI and normal birds were not different. Approximately 2 μmole of sodium were lost per gram of dry feces in both groups.

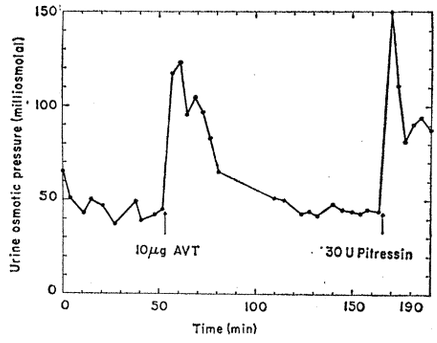

Fig. 1. The effect of intravenous injection of AVT and lysine vasopressin on the osmotic pressure of urine from the right ureter of a male DI chicken.

To investigate the expression of the DI trait at early ages, we studied three groups of chicks. In two groups, water consumption was measured in two series of 3- to 4-week-old birds whose phenotype was predicted on the basis of the parental pedigree to be either normal or DI (Table 2). In both instances the series predicted to be DI drank more water per unit body weight. The magnitude of this response was larger than in adult birds. A similar experiment on 4-week-old chicks had the same results. In the second experiment the individual birds were retested at 12 to 28 weeks of age. In most cases the size of the drinking response was less in the older birds. The two categories of normal and DI birds remained distinct, confirming the stability of the trait with age within individual animals.

To test the antidiuretic response of the DI chicken kidney, we infused four birds with 10 m*M* NaCl through the wing vein at 1.8 ml/min, and one of the ureters was catheterized with a polyethylene tube. After the urine flow became constant, Ringer solutions, aqueous lysine vasopressin (ADH), or aqueous AVT were injected intravenously. The control Ringer solutions produced no antidiuretic response; ADH and AVT produced pronounced increases in osmotic pressure of the urine (Fig. 1). Under the conditions of this experiment, no consistent changes in urine volume occurred. The mean drinking response of the four DI birds before the experiment was 23.8 ± 4.3 percent of body weight per day. A test dose of 10 μg of AVT (Sandoz) produced an average increase in urine osmotic pressure of 2.87 ± 1.26 times. The mean urine osmotic pressure increased 2.14 ± 0.57 times in response to injection of 10 μg of AVT in two normal chickens with a mean water consumption of 7.7 ± 0.5 percent of body weight per day and infused as above. To produce an increase in the urine osmotic pressure of two to three times in the DI birds, 20 to 30 units of vasopressin were required. On the basis of these results it seemed clear that the kidney of DI chickens was quite responsive to both AVT and ADH. Our data indicate that the DI chicken kidney is not more sensitive than the normal one. However, the Brattleboro rat kidney is more sensitive (*3*). The dosages of AVT used by us and by Skadhauge (*4*) are quite large compared to the probable physiological concentration. Doses as small as 13 to 25 ng cause an average increase in ureteral urine osmolality of 56 percent (*7*).

There are several possible explanations for the hereditary DI. The polydipsia may be primary, caused by a defect in the neural thirst centers resulting in compulsive drinking and secondarily in polyuria. On the other hand the polydipsia may be a secondary result of a failure in hypothalamic AVT synthesis or in a decreased ability of the kidney, intestine, or cloaca to respond to AVT release with an antidiuretic response.

The mutant condition we describe is interesting because many factors in the regulation of avian water metabolism and the control of the permeability of membranes to water are involved. With the development of this inbred strain of DI chickens, we are in a strong position for investigating the nature of the altered genotype at the molecular level.

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