the level of DDD in the liver might be useful as a diagnostic criterion.

Quantitative relations of metabolites differed among tissues as well as between dead and living birds (Table 2). In both living and dead birds, DDD residues reached their highest concentrations in the liver, and DDT, in the remainders, while DDE was at its highest concentration in the livers of dead birds and in the remainders of survivors.

It seems appropriate to add together the DDT and DDD residues, for both are commercial insecticides that are known to affect birds. Furthermore, DDD may be formed from DDT under widely varying natural conditions (*10*), including postmortem decomposition of animals (*11*). Since most animals are stored for weeks or years before being analyzed, it is possible that much of the DDT is converted to DDD after death.

The amount of DDT converted after death may vary considerably from organ to organ. For this reason, we cannot state that the concentration of metabolites found in different parts of the body (Table 2) reflects the condition in life. However, the differences are so great that they probably reflect life processes in large part. Certainly the avian liver converts a great part of the DDT to DDD, and it is logical to assume that this is not solely a postmortem effect.

DDE did not appear as a result of postmortem decomposition (*11*). This metabolite of DDT is formed rather slowly and is lost slowly; often it is found in large amounts in apparently healthy animals. Probably much of it is picked up from food organisms in which it has already been metabolized to DDE. Little is known about the effects of DDE in vertebrates, but this compound is not a commercial pesticide and is generally reported to be of low toxicity, a view that is in harmony with our findings.

The validity and usefulness of DDT residues in the brain for indicating death from DDT poisoning is strengthened by extension to additional species of birds, and particularly by the consistency of quantities in birds dying at various times after dosage; brain residue levels are seen to be similar in birds and certain mammals. Residues in livers and remainders appear to be more variable and time-related than those in brains and hence are of less value in diagnosing mortality. The concentration of DDE was low in dead specimens but was the highest metabolite in survivors. The relative importance of DDT and DDD was not apparent.

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References and Notes

1. R. F. Bernard, *Mich. State Univ., Publ. of Mus., Biol. Ser.* 2, (1963), p. 155.
2. W. E. Dale, T. B. Gaines, W. J. Hayes, Jr., G. W. Pearce, *Science* **142**, 1474 (1963).
3. W. J. Hayes, Jr., *Ann. Rev. Pharmacol.* **5**, 27 (1965).
4. D. H. Wurster, C. F. Wurster, Jr., W. N. Strickland, *Ecology* **46**, 488 (1965).
5. Tissues were dried to constant weight at 48°C, ground with sodium sulfate to a free flowing powder, extracted for 8 hours with a mixture (50 : 50) of ether and Skellysolve B in Soxhlet thimbles, and cleaned by acetonitrile partitioning and passage through a florisil column. Reading was on a Barber-Coleman 500 (Sr. 90) electron capture gas chromatograph with a column (0.64 cm by 1.2 m) packed with 57. DC-200 (12,500) oil on ABS Chromsorb W. Flow rate was 74 ml per minute; temperatures were: column, 210°C; injector, 240°C; and detector, 230°C.
6. L. B. Hunt, Final Rep., contract 14-16-0008-659 with U.S. Fish and Wildlife Service (1965), part 3.
7. Patuxent Center, unpublished data.
8. E. A. Boykins, thesis, Michigan State University (1964).
9. W. E. Dale, T. B. Gaines, W. J. Hayes, Jr., *Toxicol. Appl. Pharmacol.* **4**, 89 (1962).
10. R. P. Miskus, D. P. Blair, J. E. Casida, *J. Agr. Food Chem.* **13**, 481 (1965).
11. P. S. Barker, F. O. Morrison, *Can. J. Zool.* **42**, 324 (1964).
12. A. M. Mattson, J. F. Spillane, C. Baker, G. W. Pearce, *Anal. Chem.* **25**, 1065 (1953).
13. M. S. Schechter, S. B. Soloway, R. A. Hayes, H. L. Haller, *Ind. Eng. Chem. Anal. Ed.* **17**, 704 (1945).

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Association between Potassium Concentration and Serological Type of Sheep Red Blood Cells

Abstract. *When red blood cells from 115 sheep were classified for the presence or absence of antigenic factor M and for high (as opposed to low) potassium concentration levels, the cells of the 22 M-negative sheep were low in potassium.*

Gene *M* is responsible for the presence of the M antigenic factor of sheep red cells and the corresponding M-positive phenotype; *mm* sheep have M-negative red cells (*1*). Gene Ka^L, for low concentration of red-cell potassium (low K), is dominant to its allele ka^h so that ka^hka^h sheep are of the high-potassium (high K) type (*2*).

Table 1. Classification of red cells from 115 sheep for antigenic factor M and potassium type (low or high).

M class	Low		High
	$Ka^L/?$	Ka^Lka^h	ka^hka^h
Negative	22	0	0
Positive	22	26	45

Table 2. Genotypes and phenotypes for the M red-cell factor and for red-cell potassium concentration in sheep of three breeds and their crosses.

M negative	M positive		
mm	M(m)*	*M(m)**	*M(M)**
Low K	Low K	Low K	High K
$Ka^L(Ka^L)$*	$Ka^L(ka^h)$*	Ka^Lka^h	ka^hka^h
	Cheviot		
3	2	3	3
	Scottish blackface		
3	6	3	7
	Welsh mountain		
0	4	2	6
	Cheviot × Scottish blackface		
3	4	9	7
	Cheviot × Welsh mountain		
10	4	3	6
	Scottish blackface × Welsh mountain		
3	2	6	16

* Predicted genotype

The red cells from 115 sheep of three breeds and their crosses were classified for the M factor with an ovine antiserum (isoimmune) in a hemolytic test (*1*) and for high or low-potassium type from estimation of the red-cell potassium concentration by flame photometry (*3*) (Table 1). With no prior information to indicate an association between M types and potassium types, four different phenotypes would be expected. However, all of the 22 M-negative bloods were from sheep of the low-potassium type; none was classified as M-negative, high potassium. Furthermore, all 26 sheep previously known by progeny tests to be heterozygous (Ka^Lka^h) for low potassium were M-positive, suggesting that all M-negative sheep are homozygous (Ka^LKa^L) for low potassium.

The association between the M-negative (*mm*) genotype and the apparently homozygous (Ka^LKa^L) low-potassium genotype suggests that the relation between the notation for the possible genotypes and the corresponding phenotypes is as follows:

Genotype	Phenotype
$Ka^LKa^L = mm$	Low K, M-negative
$Ka^Lka^h = Mm$	Low K, M-positive
$ka^hka^h = MM$	High K, M-positive

This phenotypic relation occurs in populations of widely differing genetic backgrounds as shown in Table 2. The data for each of the three breeds and their crosses are in accord that *M* is equivalent to ka^h and *m* is equivalent to Ka^L, but the precise nature of this equivalence remains to be determined.

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References and Notes

1. B. A. Rasmusen, C. Stormont, Y. Suzuki, *Genetics* **45**, 1595 (1960). Red cells with either factor M or M_x are considered as M-positive.
2. J. V. Evans and J. W. B. King, *Nature* **176**, 171 (1955). Evans and King did not use gene symbols. We are using *Ka* as the base symbol for the locus responsible for the different levels of red-cell potassium. Ka^L is the dominant gene for low K, and ka^h is its recessive allele for high K.
3. J. V. Evans, *Austral. J. Agric. Res.* **14**, 540 (1963).
4. Supported in part by fellowship 1F3 HD-28, 393-O1G and D, from the NIH. Dr. G. Wiener provided information on the pedigrees of the sheep.

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Deamino-Oxytocin and 1-γ-Mercaptobutyric Acid–Oxytocin: X-ray Crystallographic Data

Abstract. *X-ray studies of crystalline deamino-oxytocin (1-β-mercaptopropionic acid–oxytocin) have established the existence of both wet and dry forms. A remarkable degree of similarity exists between the two forms even though there are changes in cell dimensions and space group on drying. The dry form of 1-γ-mercaptobutyric acid–oxytocin is very similar to that of deamino-oxytocin.*

Deamino-oxytocin (1-β-mercaptopropionic acid–oxytocin) and 1-γ-mercaptobutyric acid–oxytocin were both first crystallized from 1*N* acetic acid by Jarvis and du Vigneaud (*1*). Ferrier, Jarvis, and du Vigneaud have shown that both compounds can be crystallized from water (*2*). Deamino-oxytocin exerts some of the pharmacological activities of oxytocin to an even greater degree than does the hormone itself, whereas 1-γ-mercaptobutyric acid–oxytocin is virtually inactive.

Using a slight modification of their procedure, slow cooling over a narrower temperature range, we have obtained much larger crystals (0.4 × 0.08 × 0.01 mm) of deamino-oxytocin. These crystals are monoclinic laths, elongated along *b*, lying on (001), and frequently terminated by complex high-order forms. The uniterminal character of the diad axis is usually evident. On standing in air, the main face of the crystal loses brilliance and appears eroded. A discrete air-dried stage has been defined.

Table 1. Data for deamino-oxytocin and 1-γ-mercaptobutyric acid–oxytocin.

Form	Unit cell dimensions					Space group	Measured density (D_m) (g/ml)
	a Å	*b* Å	*c* Å	β °	*V* (10^{30} Å^3)		
			Deamino–Oxytocin				
Wet	27.3 ± .1	9.07 ± .03	23.1 ± .1	102.4 ± .3	5.60 ± .06	$P2_1$	1.305 ± .005* 1.328 ± .005†
Dry	28.1 ± .2	9.43 ± .06	24.5 ± .2	124.0 ± .3	5.38 ± .10	*C*2	1.328 ± .005
			1-γ-Mercaptobutyric acid–oxytocin				
Dry	28.0 ± .2	9.24 ± .06	24.3 ± .2	121.4 ± .3	5.35 ± .10	*C*2‡	1.319 ± .005

* Measured in aqueous column. † Measured in water-saturated xylene-bromobenzene column. ‡ More probable of two possible space groups.

We have examined only a preparation of the air-dried form of 1-γ-mercaptobutyric acid–oxytocin. The crystals were small, and many of them were fragmented. They are thin monoclinic plates, elongated along *b*, and lying on (001).

X-ray crystallographic data for both the wet and the dry forms of deamino-oxytocin and the 1-γ-mercaptobutyric acid–oxytocin were obtained from oscillation, Weissenberg, and precession photographs; CuK_α radiation was used. The densities were determined by a modification of the normal density-gradient-tube procedure (*3*). The gradient tube was viewed between crossed sheets of Polaroid in order to determine the position of the extremely small samples of the colorless crystals. By this technique, a density determination was possible with a few crystals of total weight approximately 10μg. These crystals could not be seen in the gradient tube without the aid of the crossed Polaroid sheets. The results of the x-ray and the density studies are shown in Table 1.

When deamino-oxytocin crystals are dried, there is not only a change in cell dimensions but also a change in space group from $P2_1$ in the wet form to *C*2 in the dry form. However, this change is not so significant as it might appear, since the intensity distribution of the 0*kl*, *hk*0, and *h0l* reflections of the wet form of deamino-oxytocin all showed marked pseudo face-centering related to the face-centering exhibited by the dry form.

The density of the wet deamino-oxytocin crystals was determined in both water-saturated xylene-bromobenzene and aqueous columns. The density (1.305 g/ml) obtained with the aqueous column leads to a value of 2210±40 for the molecular weight of the asymmetric unit. This corresponds to two molecules of deamino-oxytocin ($C_{43}N_{11}O_{12}H_{65}S_2$, molecular weight: 991) and 12±2 molecules of water. The density (1.328 g/ml) obtained with the water-saturated xylene-bromobenzene column leads to a value of 2242±40, corresponding to two molecules of deamino-oxytocin and 14±2 molecules of water. The air-dried form of deamino-oxytocin has a density of 1.328 g/ml which leads to an asymmetric unit weight of 1077±30, corresponding to one molecule of deamino-oxytocin and 5±2 molecules of water. In the normal transition from the wet to the air-dried state in proteins and large peptides, water is lost and there is a corresponding decrease in the volume of the crystal. The loss of some of the aqueous component of higher partial specific volume leads to an increase in density. The existence of an air-dried form with a measured density of 1.328 g/ml therefore suggests very strongly that for the wet form the lower value of the density found in the aqueous column should be considered more probable. Water could be lost from the crystals in the xylene-bromobenzene column. The volumetric change observed on drying is compatible with a loss of either three or four molecules of water per two molecules of deamino-oxytocin (the asymmetric unit in the wet form).

The wet crystals of deamino-oxytocin give good diffraction patterns. The minimum spacing observed was 1.1 Å. The dry crystals are markedly disordered with a minimum spacing of 1.8 Å. These crystals are highly mosaic with