tozoa did so much less effectively. Semen from the three species was incubated with 1–4 μg of tetracycline HCl for each 10^6 spermatozoa. The spermatozoa were washed several times at 3–6,000 r.p.m. for 15 min and then checked for fluorescence. Labelled spermatozoa centrifuged at the higher speeds lost some of their fluorescence; however, in all cases the fluorescence was still very noticeable. Rabbit semen was labelled with 4 μg/10^6 spermatozoa of the antibiotic, separated from the seminal plasma, and then incubated at 37° C for 8 h in 'Tyrode' solution with sodium pyruvate added. Motility decreased from 80 per cent to 10 to 20 per cent during the 8 h period but spermatozoa fluorescence was not altered. Likewise, rabbit spermatozoa shaken for 12 h at room temperature did not diminish in fluorescence.

Tetracyclines had previously been found in human semen, and we had established the *in vitro* binding of tetracycline HCl to human, boar, and rabbit spermatozoa, and so it seemed probable that this antibiotic would bind also *in vivo*. A series of experiments with rabbits was performed to establish this concept. Semen was collected with an artificial vagina before treatment to obtain values on normal semen parameters[8]. Dutch Belted and New Zealand males received intravenously 25 mg/kg of tetracycline HCl twice a day for 2 days. Control animals received sterile water with 0·25 per cent methylcellulose vehicle only. Twenty rabbits were used in various experiments and no fluorescent spermatozoa were seen in any pretreatment or control semen samples. Also, no detrimental effects, resulting from treatment, were found in spermatozoa motility, numbers or morphology.

Semen from treated rabbits was collected 2–4 h after treatment and generally found (73 per cent of the rabbits) to contain spermatozoa which fluoresced. It is interesting that frequency and time of semen collection before treatment seem involved. Rabbits which were ejaculated seven times on the day treatment started all had good post-treatment fluorescent spermatozoa, whereas rabbits which had not been ejaculated from 1–14 days before the beginning of treatment had post-treatment spermatozoa which ranged from no fluorescence to good fluorescence. Cauda epididymal spermatozoa from treated males were checked and found not to fluoresce (these spermatozoa did fluoresce, however, when placed with the antibiotic *in vitro*). If spermatozoa do not pick up tetracycline HCl in the epididymis, then the accessory glands must trap it.

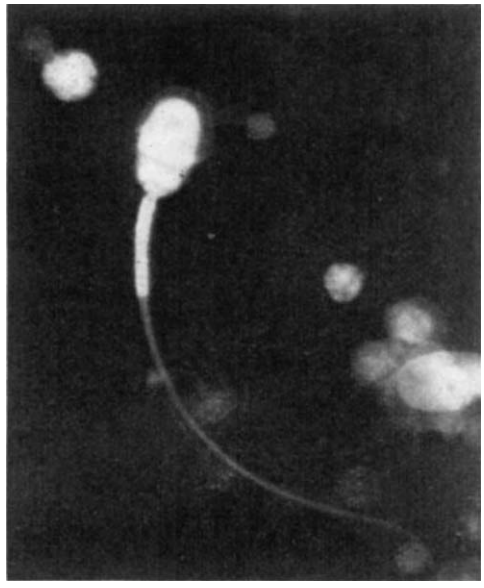

Fig. 1. Darkfield fluorescent photomicrograph of an aged rabbit spermatozoon labelled *in vitro* with tetracycline HCl. Note the partial detachment of the outer acrosome. (× 960.)

The glandulae vesiculares were found to fluoresce in rabbits treated with the antibiotic. This may explain why males from which one or two ejaculates had been collected several days before treatment did not consistently have good fluorescent spermatozoa in the post-treatment semen samples. The accessory glands from these males were most likely not as active in their secretory function as those glands from males whose seminal fluid stores had been exhausted immediately before treatment.

Rabbit spermatozoa labelled with tetracycline HCl *in vivo* did not fluoresce as brightly as those labelled with 3–4 μg/10^6 spermatozoa *in vitro*. The pattern of fluorescent intensity on different regions of a spermatozoon was, however, the same; non-motile spermatozoa fluoresced somewhat brighter than motile ones. Washing spermatozoa several times decreased the fluorescence but did not remove all of it. Labelled and non-labelled spermatozoa survived equally well when stored at 5° C for 48–60 h.

Washed ejaculated or epididymal spermatozoa from control males were suspended in urine from treated males and they immediately fluoresced. As tetracycline HCl is excreted through the urine[9], the urethral lumen (through which semen must pass) would be contaminated with the antibiotic. The first post-treatment semen sample served to flush out the urethra and then fluorescent determinations were made on the second and third semen samples collected. As the fluorophor was seen bound to spermatozoa in these last two ejaculates, its source had to be the semen and not a urine contaminant.

Evidence so far indicates that the attachment of antibiotic and spermatozoa is not detrimental to the motility, morphology or concentration of spermatozoa. This technique seems ideal for investigating the morphological differences of fresh and aged spermatozoa of the same and different species. The *in vivo* labelling of spermatozoa with tetracycline HCl apparently occurs at ejaculation, as epididymal spermatozoa do not show fluorescent evidence of the drug. Perhaps the concentration of the antibiotic in seminal fluids can be altered if the accessory glands are actively secreting during drug administration.

We thank Dr. D. Buthala for help with the photomicrography.

R. J. Ericsson
V. F. Baker

Biochemical Research,
The Upjohn Company,
Kalamazoo, Michigan.

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*N*δ-(2-amino-2-carboxyethyl)-ornithine, a New Amino-acid from Alkali-treated Proteins

A new amino-acid, *N*ε-(2-amino-2-carboxyethyl)-lysine, in acid hydrolysates of alkali-treated proteins has been reported[1,2]. The trivial name lysinoalanine was chosen for this compound, which is probably formed by the addition of an amino group of a lysyl residue at the double bond of a dehydroalanyl residue formed after alkaline decomposition of disulphide bonds. Lysinoalanine has been synthesized by two different methods[1,3] in order to confirm its chemical structure.

Further investigations of hydrolysates of wool samples including sericin treated with alkali have revealed a new amino-acid on the short column of the 'Spinco' automatic amino-acid analyser[4]. This new amino-acid, obtained

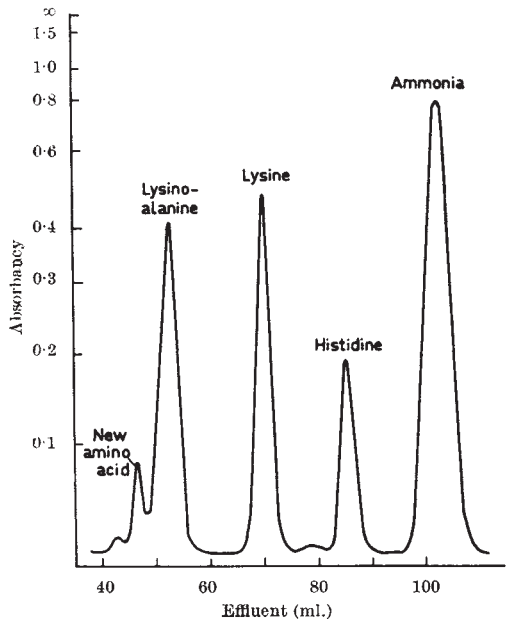
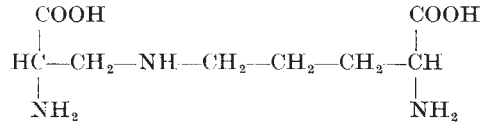


Fig. 1. Presence of a new amino-acid in an acid hydrolysate of sericin treated with alkali. (Elution patterns from the 15 cm column[4].)

from an acid hydrolysate of sericin treated with alkali, emerged before lysinoalanine as shown in Fig. 1. Merino wool, denatured in 8 molar lithium bromide at 100° C for 90 min was treated for 16 h in a solution of 0·7 molar potassium carbonate at 50° C (ref. 5), washed with water and finally dried before analysis.

Sericin, isolated from silk (*Bombyx mori*) by heating in water at 90° C, was boiled with 0·1 molar sodium carbonate for 60 min. Most of the salt could be removed by dialysis for 24 h. The solution was evaporated to dryness, and the proteins were hydrolysed with 6 normal hydrochloric acid in sealed tubes in a vacuum.

By comparing the amino-acid composition of untreated and alkali-treated sericin, loss of serine, threonine, lysine and a small decrease of arginine have been observed. It is well known that combined or free arginine is degraded by weak or strong alkalis to form ornithine and (or) citrulline. If we assume that δ-amino groups of combined ornithine could react with dehydroalanine residues, present as decomposition products of cystine and (or) serine residues, a new compound could be formed as an artefact. By analogy to lysinoalanine it should be named ornithinoalanine. Its chemical structure could be the following



In order to support our assumption we have prepared ornithinoalanine by the addition of $N\alpha$-acetyl-L-ornithine to the double bond of *N*-acetyl-dehydroalanine-ethylester, a procedure similar to that described for the preparation of lysinoalanine[3]. The resulting product of $N\delta$-(2-acetamino-2-ethoxycarbonyl-ethyl)-$N\alpha$-acetyl-ornithine was treated with 6 normal hydrochloric acid to remove the two protecting acetyl groups and the one ester group. We have obtained colourless crystals contaminated by traces of ornithine. After several recrystallizations from water by adding ethanol, ornithinoalanine was obtained as a slightly hygroscopic material with a yield of 1·1 g (37·6 per cent of theoretical value). The molecular weight was 273·72, and the formula $C_8H_{17}N_3O_4.HCl.H_2O$. As percentages; calculated: carbon 35·10, hydrogen 7·36, nitrogen 15·35, chlorine 12·95; found: carbon 35·61, hydrogen 7·09, nitrogen 14·95, chlorine 15·26.

Ornithinoalanine has been further characterized by preparing its picrate at *p*H 8–9; melting point, 185°–188° C. The electrophoretic mobility of ornithinoalanine on paper-electrophoresis was compared with other naturally occurring basic amino-acids (Table 1).

Table 1. ELECTROPHORETIC MOBILITIES OF AMINO-ACIDS ON PAPER ELECTROPHORESIS (150 MIN, 40 V/CM, IN PYRIDINE–ACETIC ACID-WATER, *p*H 6·2)

	Distance of migration towards cathode (cm)
Ornithine	21·7
Lysine	20·0
Arginine	16·6
Ornithinoalanine	11·5
Lysinoalanine	10·5

The value of the integration constant on the automatic amino-acid analyser was found to be 1·29 times that of lysine.

Table 2. FORMATION OF ORNITHINOALANINE AND LYSINOALANINE IN SERICINE TREATED WITH ALKALI

	Amino-acid (μmoles/g) found in sericin	
	Untreated	Boiled in 0·1 molar sodium carbonate for 60 min
Lysine	210	141
Lysinoalanine	–	75
Arginine	255	220
Ornithinoalanine	–	23
Serine	349	206

From the amino-acid data given in Table 2 it can be concluded that ornithinoalanine is accounted for by more than half the arginine that disappears during treatment of sericin with alkali. The significant decrease of 143 μmoles/g of serine indicates its degradation by alkali to dehydroalanine residues which react in such a way that 23 μmoles/g of ornithinoalanine are formed. This confirms other results[5] according to which serine is responsible for providing dehydroalanine residues to react with lysine to form lysinoalanine.

The amount of ornithinoalanine in hydrolysates of denatured and subsequently alkali-treated wool is about 10 μmoles/g. Investigations to find the conditions which will give larger yields of this new amino-acid are in progress.

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KL. ZIEGLER
I. MELCHERT
C. LÜRKEN

Deutsches Wollforschungsinstitut
an der Technischen Hochschule,
Aachen.

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Multiple Analyses on a Single Disc Electrophoretic Preparation

DISC electrophoresis has been shown to be a rapid means of producing very high resolution separations of proteins[1,2]. Various histochemical methods, such as the periodic acid–Schiff and the Feulgen techniques, have been applied to the gel columns to identify various proteins after electrophoretic separation. Although the reproducibility of disc electrophoresis is quite good, it is difficult to correlate results of staining each of several replicate separations by different methods because one cannot be completely certain that each protein fraction migrated exactly the same distance in each replicate gel column, and various