Table 1. The Content of Anserine, Carnosine, Histidine and N-methylhistidine of Red and White Muscles from a Number of Animal Species

Animal	Muscle	Anserine (μmol./gm.)	Carnosine (μmol./gm.)	Histidine and N-methyl-histidine (μmol./gm.)
Rabbit	Longissimus dorsi	19·3	3·2	0
	Psoas	21·8	2·3	0
	Heart	0	0	0
	Semi-tendinosus	Trace	0	0
	Liver	0	0	0
Pigeon	Pectoral	4·4	1·0	0
	Leg	0	0	0
Sperm whale	Longissimus dorsi	4·9	9·1	0
Horse	Longissimus dorsi	0	25·5	0
Chicken	Pectoral	43·5	12·3	0
	Leg	7·4	2·2	0
Rat	Longissimus dorsi	8·9	3	0
	Gastroc-nemius	6·7	0	0
	Heart	0	0	0

The most significant feature of the results is that the so-called white muscles, for example, the pectoral of the fowl and the psoas and longissimus dorsi of the rabbit, contain large amounts of anserine and little carnosine, whereas red muscles such as the pectoral of the pigeon and semitendinosus of the rabbit contain little of either dipeptide. The extreme case of a red muscle is, of course, the heart, which is better supplied with blood than other muscles. Rat and rabbit heart contain no detectable amounts of carnosine or anserine. The longissimus dorsi of the horse is exceptional in having a high level of carnosine, but no anserine, while the considerably whiter muscles of the rat contain little anserine and almost no carnosine. There is a relatively low concentration of the dipeptides in the longissimus dorsi of the sperm whale. In no case are the precursors histidine and N-methylhistidine present in detectable amounts. It is interesting that the peak which contains the neutral and acidic amino-acids (Fig. 1) is 8–10 times larger in muscles containing little or no carnosine and anserine than for muscles rich in these dipeptides. The high concentration of these amino-acids in muscles such as rat heart is of the same order as the concentration of the dipeptides in white muscle such as rabbit psoas.

The results, which are particularly interesting in view of the recent importance attached to carnosine and anserine in both aerobic and anaerobic glycolysis[5,6,8], are being followed up with a detailed study of the intermediary metabolism of these dipeptides.

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Oct. 16.

[1] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **192**, 663 (1951).
[2] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **211**, 893 (1954).
[3] Davies, C. W., *Biochem. J.*, **45**, 38 (1949).
[4] Jones, N. R., *Biochem. J.*, **60**, 81 (1955).
[5] Yudaev, N. A., Smirnov, M. I., Razina, P. G., and Dobbert, N. M., *Biokhimiya*, **18**, 732 (1953).
[6] Meshkova, N. P., and Zaitseva, N. N., *Doklady Akad. Nauk. S.S.S.R.*, **92**, 999 (1953).
[7] Goodall, M. C., *Nature*, **178**, 540 (1956).
[8] Yemm, E. W., and Cocking, E. C., *Analyst*, **80**, 209 (1955).

Interconversion of 17-Keto and 17β-Hydroxyl Groups in Steroids by Protozoa

It has been amply demonstrated that bacteria, yeasts, moulds and actinomycetes convert steroids in various ways. In many instances, the products obtained by such microbial action have been identified and range from modifications at one or two carbon atoms (hydroxylation, dehydrogenation, etc.) to a complete degradation of the steroidal molecule[1]. However, almost nothing is known about the ways in which such compounds are affected by micro-organisms of the animal kingdom. It has been reported that in some cases Protozoa require steroidal materials for their subsistence[2], but the identity of their metabolic intermediates or products has not been established. We wish to report that a specific and well-defined reaction can be brought about by two protozoan species of the genus *Trichomonas*.

Cultures of *Trichomonas gallinae* were grown in 100 ml. of a trypticase – serum – thioglycollate medium for 24 hr. at 37° C. At the end of this period 10 mgm. of the steroid to be tested was added to the culture and the incubation continued for an additional 24 hr., either under anaerobic conditions or aerated in shaken flasks. The steroidal material was then extracted from the culture medium with methylene chloride and identified by paper chromatography in six solvent systems[3].

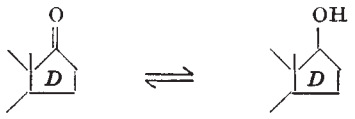
The results indicated that all the 17-ketosteroids tested were reduced to their corresponding 17β-hydroxy analogues in an anaerobic environment, and that a reversal of such reductions occurred under aerobic conditions (Table 1).

Table 1. Conversions of Steroids by *Trichomonas gallinae* 10 mgm. of steroid dissolved in 1 ml. propylene glycol/100 ml. medium. One-day growth and one-day conversion at 37° C.

Reaction	Substrate	Product
Reduction	4-Androstene-3,17-dione	17β-Hydroxy-4-androsten-3-one
	4-Androstene-3,11,17-trione	17β-Hydroxy-4-androstene-3,11-dione
	1,4-Androstadiene-3,17-dione	17β-Hydroxy-1,4-androstadien-3-one
	11α-Hydroxy-4-androstene-3,17-dione	11α,17β-Dihydroxy-4-androsten-3-one
	11β-Hydroxy-4-androstene-3,17-dione	11β,17β-Dihydroxy-4-androsten-3-one
	3β-Hydroxy-5-androsten-17-one	5-Androstene-3β,17β-diol
	3β-Acetoxy-5-androsten-17-one	3β-Acetoxy-5-androsten-17β-ol + 3β-Hydroxy-5-androsten-17-one + 3β-Hydroxy-5-androsten-17β-ol
	Œstrone	Œstradiol
Oxidation	17β-Hydroxy-4-androsten-3-one	4-Androstene-3,17-dione
	11β,17β-Dihydroxy-4-androsten-3-one	11β-Hydroxy-4-androstene-3,17-dione
	17β-Hydroxy-4-androstene-3,11-dione	4-Androstene-3,11,17-trione
	17β-Hydroxy-4-œstren-3-one	4-Œstren-3,17-dione

In addition, the bioconversion product of 4-androstene-3,11,17-trione was isolated by column chromatography and identified as 17β-hydroxy-4-androstene-3,11-dione (11-ketotestosterone) by mixed melting point with the corresponding authentic steroid and by infra-red analysis. No transformations by this organism of Δ^4-3-keto-pregnene derivatives were detected.

These results show that the trichomonad contains a dehydrogenase which carries out a specific interconversion of the 17-keto and 17β-hydroxyl groups in C_{18} and C_{19} steroids:



We have observed that another trichomonad (*T. foetus*) also brings about specific transformations of this type.

The interconversion of keto- and hydroxy-steroids occurs in a variety of mammalian tissues[4]. In many instances, bacteria[5], yeasts[6], moulds[7] and actinomycetes[8] have also been reported to affect the oxygenation of C_{18} and C_{19} steroids in the 17- as well as the 3-positions. Our findings show that reactions of this type can also be carried out by Protozoa.

Further work will be published elsewhere. Acknowledgment is made to Mr. L. M. Reineke and his associates for the paper chromatographic analyses, and to Dr. J. L. Johnson and Mrs. G. S. Fonken for the infra-red spectrographic data.

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[1] Hanč, O., and Riedl-Tůmová, E., *Pharmazie*, **9**, 877 (1954). Wettstein, A., *Experientia*, **11**, 465 (1955). Eppstein, S. H., Meister, P. D., Murray, H. C., and Peterson, D. H., in "Vitamins and Hormones", **14**, 359 (Academic Press, New York, N.Y., 1956).

[2] Cf. Rees, C. W., Bozicewich, J., Reardon, L. V., and Daft, F. S., *Amer. J. Trop. Med.*, **24**, 189 (1944). Conner, R. L., and van Wagtendonk, W. J., *J. Gen. Microbiol.*, **12**, 31 (1955). Vishniak, H. S., *J. Gen. Microbiol.*, **12**, 464 (1955).

[3] Reineke, L. M., *Anal. Chem.*, **28**, 1853 (1956).

[4] Cf. Lieberman, S., and Teich, S., *Pharmacol. Rev.*, **5**, 285 (1953).

[5] Mamoli, L., Koch, R., and Teschen, H., *Z. physiol. Chem.*, **261**, 287 (1939). Cf. Arnaudi, C., *Applied Microbiol.*, **2**, 274 (1954). Marcus, P. I., and Talalay, P., *J. Biol. Chem.*, **218**, 661 (1956).

[6] Mamoli, L., and Vercellone, A., *Z. physiol. Chem.*, **245**, 93 (1937). Wettstein, A., *Helv. Chim. Acta*, **22**, 250 (1939). Butenandt, A., Dannenberg, H., and Surányi, L. A., *Ber.*, **73**, 818 (1940).

[7] Cf. Vischer, E., and Wettstein, A., *Experientia*, **9**, 371 (1953).

[8] Turfitt, G. E., *Biochem. J.*, **40**, 79 (1946); **42**, 376 (1948). Heusghem, C., and Welsch, M., *Bull. Soc. Chim. Biol.*, **31**, 282 (1949).

Isolation of Melanin Granules

THE study of melanin granules is of both commercial and academic importance in the investigation of the pigmentation and bleaching of animal fibres. A means of separation of the granules from the keratin matrix which leaves them morphologically and chemically unaffected is therefore desirable. The methods of isolation which have so far been used consist in dissolving the keratinous material either by acid or alkaline hydrolysis, or by treatment with a mixture of phenol hydrate and thioglycollic acid. Of these, the last[1,2] appears to be the most satisfactory, because it leaves the granules undamaged. It is, however, extremely laborious, involving refluxing of the protein substance for twenty-four hours, after which the resultant suspension is centrifuged for 10 hr.

By refluxing melanin-containing protein with 60 per cent w/v hydrazine/absolute alcohol solution for 2 hr., using a liquor ratio of 50 : 1, it has been found possible to isolate the pigment granules. The protein is converted into hydrazides of low molecular weight[3,4] and any undispersed matter is filtered out on a coarse filter paper, through which the granules pass. The suspension is centrifuged for 2 hr. at 2,000 *g*, the supernatant liquor is decanted off and the residual granules are washed three times with distilled water.

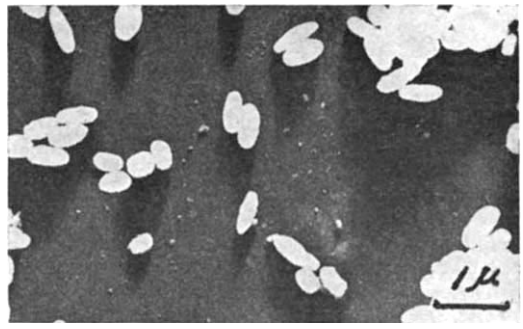

Fig. 1

The accompanying electron micrograph (Fig. 1), for which we are indebted to Dr. J. Sikorski, shows granules which have been isolated by the above treatment from dark-brown Welsh mountain wool. As may be seen, they appear to be morphologically unaffected, though in this instance they were refluxed for 4 hr. Even after digestion for 2 hr., however, the granules are quite clean, and no advantage is to be gained by the longer treatment.

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[1] Laxer, Sikorski, Whewell and Woods, *Biochim. Biophys. Acta*, **15**, 174 (1954).

[2] Hope, Sikorski and Whewell, Proc. First European Regional Conference on Electron Microscopy, Stockholm 1956 (in the press).

[3] Akabori, Ohno and Norita, *Bull. Soc. Chim. Japan*, **25** (3), 214 (1952).

[4] Blackburn and Lee, *J. Text. Inst.*, **45**, *T*148 (1954).

Interatomic Bonding in Manganese Trifluoride

THE monoclinic unit cell of manganese trifluoride has dimensions *a*, 8·904 ± 0·003; *b*, 5·037 ± 0·002; *c*, 13·448 ± 0·005 A.; β, 92·74 ± 0·04°, and contains twelve manganese and thirty-six fluorine atoms in general and special positions[1] of space group $C2/c - C^6_{2h}$. The structure is pseudo-rhombohedral, and since the packing of fluorine atoms is midway between close-packed hexagonal and a ReO_3-type defective cubic close-packing, it is classified as a VF_3-type transition-element trifluoride[2]. MnF_6 octahedra are joined by sharing corners, and the lower symmetry of the structure, in comparison with other trifluorides of the first long period, results from the unusual occurrence of three different Mn–F bond-lengths (2·09, 1·91 and 1·79 A.) within each octahedron.

Crystal-field (or ligand-field) theory[3] has been applied recently by Harris, Nyholm and Stephenson[4] to explain the abnormally long Pd–I bonds observed in the distorted octahedral complex $Pd(diarsine)_2I_2$. A similar explanation is now offered for the unsymmetrical bonding in manganese trifluoride.

The magnetic moment[5] of 4·9 Bohr magnetons implies that there are four unpaired electrons in the 3*d* shell of the Mn(III) atom. Three of these occupy the d_ε orbitals, and the remaining electron is probably in a $3d_{z^2}$ orbital. The empty $3d_{(x^2-y^2)}$ orbital points in the direction of four fluorine ions and together with the 4*s* and two 4*p* orbitals forms four hybrid dsp^2 bonds directed towards the corners of a square. The three singly occupied d_ε orbitals offer no repulsion