

photograph would be one of practically normal well-oriented  $\alpha$ -keratin, and it must be concluded, both on this account and also because of the relatively unimportant entropy effect, that the mechanism of contraction down to some 20 per cent below the initial length is not one involving random disorientation.

It may be noted in Figs. 1 and 2 that supercontracted keratin exhibits a finite positive entropy effect at the lowest extensions. In this respect it differs from rubber (and, indeed, from most other high polymers) and may be classed with nylon as being more 'rubber-like' than rubber itself at low extensions!

Experiments carried out with films of the muscle protein, myosin, oriented by the technique described by Astbury and Dickinson<sup>4</sup>, show that, although the elastic properties of myosin bear a marked resemblance to those of 'generalized' keratin (myosin, however, is even more susceptible to the action of hot water), the entropy effects in normal and supercontracted myosin are more like those in normal keratin: the entropy increases slightly during extensions up to some 20 per cent at least, although there is sometimes to be observed an initial decrease at the lowest extensions, followed by an increase. (The use of the term 'normal' here is not strictly correct, since the experimental procedure is such as to necessitate a certain degree of relaxation. For example, in keratin at low extensions, the total load in these experiments is about 75 per cent of that obtained by quick stretching.) The elastic mechanism in myosin must thus be one involving chiefly the internal energy. The importance of these results in connexion with theories of muscle contraction have been discussed by Astbury<sup>5</sup>.

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<sup>1</sup> For example, Meyer, "High Polymers", 4 (1942).

<sup>2</sup> Bull, *J. Amer. Chem. Soc.*, **67**, 533 (1945).

<sup>3</sup> Astbury and Woods, *Phil. Trans.*, A, **232**, 33 (1933).

<sup>4</sup> Astbury and Dickinson, *Proc. Roy. Soc.*, B, **129**, 307 (1940).

<sup>5</sup> Royal Society, Croonian Lecture, 1945.

### Presence of a $\Delta^5$ -Unsaturated Sterol Derivative in the Medulla Cells of Keratin Fibres

KERATIN differs from other scleroproteins in containing a relatively high percentage of sulphur, and it is known that the sensitivity of wool and hair to attack by alkali and alkaline reducing agents results from fission of the cystine disulphide linkages, followed, under more drastic conditions of treatment, by hydrolysis of peptide linkages<sup>1,2</sup>. The medulla of sheep wool and porcupine quill, however, is devoid of cystine<sup>3</sup>, while the medulla cells of goat hair resist hydrolysis by 4*N* sodium hydroxide at room temperature, and contain little, if any, sulphur<sup>4</sup>. This observation has been extended to hair from several carnivores and rodents<sup>5,6</sup>, and would appear to be a general property of medullary keratin. The X-ray diffraction pattern of medulla cells dissected from Canadian porcupine quill reveals the presence in the cells of  $\beta$ -keratin, together with unidentified material<sup>7</sup>. On X-ray examination, medulla cells isolated from hair by treatment with cold sodium hydroxide showed no spacing corresponding to  $\beta$ -keratin; but a strong meridional arc was found, identical with that from the non-protein constituent of porcupine quill medulla cells<sup>8</sup>.

Earlier attempts to identify the non-protein constituent of medulla cells were unsuccessful<sup>9</sup>. More recently, however, experiments using bleached horse hair (South American mane), and fibres from the winter coat of the hare (*Lepus variabilis*), have yielded further information. Fibres were extracted with alcohol, ether and distilled water, and the medulla cells isolated by treatment with 4*N* sodium hydroxide for eighteen hours at room temperature, or by refluxing the fibres with 10 per cent sodium sulphide for two hours, followed by repeated washing with distilled water in a centrifuge tube.

Several drops of an aqueous suspension of the cells were pipetted on to a microscope slide, and one drop of 1 per cent tannic acid added. The cells diminished in size and the surface developed a puckered appearance. When the test was repeated using solutions of 0.1 per cent sodium oleate, or 1 per cent saponin, the cells remained unaltered in size; but the surface became covered with micro-blisters. While the possible occurrence of complicating cell permeability effects must not be overlooked, these results suggest that the surface structure of medulla cells may contain a lipo-protein-cholesterol combination<sup>8</sup>.

Rudall's experiments on hair medulla cells<sup>7</sup>, together with the fact that, unlike cross-sections of normal fibres, chemically isolated medulla cells do not give a positive diazo-reaction for tyrosine and histidine<sup>6</sup>, suggest that considerable modification of the medullary protein occurs during the process of isolation. Furthermore, from the method of isolation, it follows that if the cell wall of isolated cells contains saponifiable lipid it must be in close association with protein or with non-saponifiable lipid. The possible occurrence of sterol-protein combinations in blood<sup>10</sup>, and yeast<sup>11</sup>, together with the statement that ether extraction in a Soxhlet removes only part of the cholesterol from sheep wool<sup>12</sup>, suggested that medulla cells may contain a sterol-protein complex. Uncombined cholesterol does not appear to be present, since a chloroform extract of dry medulla cells gave a negative Liebermann-Burchard reaction. In order to test for the presence of combined sterol, medulla cells were heated with an excess of 7.5 per cent alcoholic sodium hydroxide for two hours on a steam bath; a method used by Schoenheimer and Breusch for liberating cholesterol from a wide range of biological tissues. A chloroform extract of the insoluble residue gave a negative reaction with acetic anhydride-concentrated sulphuric acid. Medulla cells were then refluxed for five hours with 10 per cent alcoholic sodium hydroxide, and the residue extracted with chloroform. When tested with the Liebermann-Burchard reagent, the chloroform extract gave a positive colour reaction, thereby showing the presence of a  $\Delta^5$ -unsaturated sterol, which is very probably cholesterol.

Preliminary tests carried out on the alcoholic sodium hydroxide extract of the medulla cells gave the following results:

On dilution with an equal volume of distilled water a white precipitate was formed. After removing the precipitate, the filtrate gave:

(1) A positive xanthoprotein reaction, a precipitate separating when ammonium hydroxide was added;

(2) Negative reactions to tests for arginine, tyrosine and tryptophane;

(3) A slight precipitate on acidification with hydrochloric acid;

(4) A positive tartaric acid-ammonium molybdate-benzidine reaction for the phosphate radical.

Further experiments are in progress and details will be published elsewhere. I am indebted to Drs. A. E. Alexander, K. M. Rudall and J. H. Schulman for helpful discussion.

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<sup>7</sup> Rudall, K. M., Thesis, University of Leeds (1936).  
<sup>8</sup> Rideal, E. K., and Schulman, J. H., *Nature*, **144**, 100 (1939).  
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<sup>10</sup> McCorkquodale, D. W., Steenbock, H., and Adkins, H., *J. Amer. Chem. Soc.*, **52**, 2512 (1930).  
<sup>11</sup> Schoenheimer, R., and Breusch, F., *J. Biol. Chem.*, **103**, 439 (1933).

### Transplantation of Larval Ovaries in *Drosophila* from and to Individuals Susceptible to Carbon Dioxide

THE carbon dioxide susceptibility in *Drosophila*, discovered by L'Héritier and Teissier<sup>1</sup>, and apparently inherited in a non-Mendelian manner, shows maternal as well as slight paternal inheritance, and has been tentatively ascribed to a parasite, perhaps a virus<sup>2</sup>. The following experiments were designed to test whether the material basis of susceptibility shows one of the properties of viruses, namely, the ability to be transmitted by grafting. This was done by using the transplantation technique of Ephrussi and Beadle<sup>3</sup>.

Larval ovaries from a stock homozygous for the recessive ebony, and showing the carbon dioxide susceptibility, were grafted into female larvae from a resistant wild-type stock. In a small percentage of cases, those primordia joined up with the genital tract of the host and became functional, either supplanting one of the two ovaries there, or joining the ducts in addition to them. Implanted females never acquired susceptibility. When mated to males homozygous for ebony, but not showing the susceptibility, such females produced offspring of two kinds: phenotypically wild-type flies which were always carbon dioxide resistant, and ebony coloured flies from the implanted ovary, which were always carbon dioxide susceptible. Table 1 shows some numerical results.

Table 1. Number of resistant and carbon dioxide susceptible offspring from 4 crosses between  $\frac{1}{2}$  resistant females carrying a functional implanted ovary from an  $\frac{e}{e}$  susceptible stock and  $\frac{e}{e}$  resistant males.

Resistant		Susceptible	
$\frac{e}{e}$	$\frac{+}{+}$	$\frac{e}{e}$	$\frac{+}{+}$
—	60	44	—
—	81	47	—
—	48	48	—
—	25	38	—

Transplantation of resistant wild-type ovaries into female larvae from a susceptible stock homozygous for ebony sometimes weakened the susceptibility of the flies emerging. The offspring of crosses between such females and resistant ebony males was again of two phenotypes, ebony and wild. But whereas the latter were never found to be carbon dioxide susceptible, the former were of two types: they were either composed of resistant and susceptible individuals, or entirely resistant (see Table 2). The cause of susceptibility being lost in the host and her offspring is not yet known.

Table 2. Number of resistant and carbon dioxide susceptible offspring from 4 crosses between females from an  $\frac{e}{e}$  carbon dioxide susceptible stock carrying a functional implanted ovary from a  $\frac{1}{2}$  resistant stock and  $\frac{e}{e}$  resistant males.

Resistant		Susceptible	
$\frac{e}{e}$	$\frac{+}{+}$	$\frac{e}{e}$	$\frac{+}{+}$
9	26	13	—
15	32	21	—
22	24	—	—
33	26	—	—

It might be argued that something which could be called a virus is lacking in the carbon dioxide susceptible stock and present in the normal. This argument, however, is not supported by the outcome of crossing between resistant females and susceptible males, where part of the offspring is susceptible. Furthermore, it has been possible to show that the susceptibility can be destroyed by excessive heat or cold. This would mean that the hypothetical 'virus' characteristic of the normal resistant type can be created at will. This seems very improbable. It seems more likely that there is some agent present in the susceptible flies and absent in the normal flies inhibiting the normal recovery from carbon dioxide narcosis in the former.