cases the sulphur atoms permit the synthesis of unique molecular types, but it is probably the molecule as a whole and not some special attribute of the sulphur atoms which is responsible for the biological effect.

SUMMARY

A series of xanthic, dithiocarbamic, thiocyanic and isothiocyanic acid derivatives, together with some oxygen analogues and benzthiazole derivatives,

have been prepared and their relative fungistatic activities recorded. In many of the compounds the sulphur atoms can be replaced by oxygen without marked loss of fungistatic power. The lower xanthates and certain substituted arylisothiocyanates have very high fungistatic activities.

The authors are indebted to several colleagues for the preparation of certain compounds, for helpful discussions and also for the carrying out of the biological testing.

REFERENCES

- Böhringer (1929). G.P. 485059.
 Davies, W. H. & Sexton, W. A. (1944). *J. chem. Soc.* p. 11.
 Everitt, E. L. & Sullivan, M. X. (1940). *J. Wash. Acad. Sci.* **30**, 125.
 Goldsworthy, M. C., Carter, R. H. & Green, E. L. (1942). *Phytopathology*, **32**, 497.
 Goldsworthy, M. C., Green, E. L. & Smith, M. A. (1942). *Phytopathology*, **32**, 6.
 Grasselli (1932). B.P. 407691.
 Roberts, J. W. (1942). *J. industr. engng Chem.* **34**, 497.
 Templeman, W. G. & Sexton, W. A. (1945). *Nature, Lond.*, **156**, 630.
 Tisdale, W. H. & Flenner, A. L. (1942). *J. industr. engng Chem.* **34**, 501.
 Walker, J. C. (1937). *Phytopathology*, **27**, 142.
 Walker, J. C., Morell, S. & Foster, H. H. (1937). *Amer. J. Bot.* **24**, 536.
 Wilcoxon, F. & McCallan, S. E. A. (1935). *Contr. Boyce Thompson Inst.* **7**, 333.

The Composition of Bovine Cervical Mucins and their Reaction with Oxidizing Agents

By E. BOYLAND, *Veterinary Laboratory, Ministry of Agriculture and Fisheries, New Haw, Weybridge*

(Received 17 December 1945)

Woodman & Hammond (1925) found that the appearance of bovine cervical mucin differs according to the physiological condition of the cow, and suggested that the nature of the mucin could be used as a guide to the reproductive state of the animal. The physical properties of the three types of vaginal mucin have been investigated by Scott Blair and his colleagues (Scott Blair, Folley, Malpress & Coppen, 1941; Scott Blair, Cowie & Folley, 1942), who have shown that the mucin of pregnant animals is stiff and plastic rather than elastic; that of oestrus shows a particular type of elasticity, while the dioestrous mucin is viscous but not elastic. The degree of flow elasticity can be measured with the 'oestroscope' and used for diagnosis of oestrus in cows. The mucin present in oestrus appears to contain very little protein and is readily differentiated from the mucins of dioestrus and pregnancy. No easy chemical test differentiating the two types of mucin, which might serve as a pregnancy diagnosis reaction, has been found.

Pathogenic organisms infecting the uterus and vagina of animals may be protected by the mucin

present which, being viscous, slows down the diffusion of drugs. Drugs applied to the genital tract would penetrate more rapidly to infecting organisms if the viscosity of the mucin were decreased. The mucins can be dissolved by oxidizing agents particularly hypochlorite. In the present work a study has been made of some chemical properties of these mucins.

EXPERIMENTAL

Each sample of mucin was obtained from the vicinity of the cervix of a normal cow. Oestroscope determination and microscopical examinations were carried out on all samples, which were discarded if they contained an excessive number of cells. Mucin was stored in the refrigerator without added preservative. The dioestrous and pregnancy samples were pooled from samples taken on different days, but mucin from different cows was not mixed. The amount of mucin obtained from cows in oestrus (20–50 ml.) was usually sufficient for many determinations to be made on a single sample.

The chemical nature of cervical mucins

The data (Table 1) are comparable with those given by Scott Blair *et al.* (1941) but not with those of Woodman & Hammond (1925), as the latter's values were obtained on post-mortem specimens which appeared to contain less water than material obtained from living animals.

Solubility of cervical mucins

Determination of the viscosity of the mucins could not be used to follow their reactions with reagents owing to the difficulty of mixing mucins with aqueous solutions. When small volumes of mucin are allowed to stand in the presence of excess of water with occasional stirring they dissolve very

Table 1. *Constituents of bovine cervical mucins*

Cow no.	...	...	...	804	793	804	318	564
Condition	...	...	...	oestrous	oestrous	dioestrous	dioestrous	pregnant
Oestroscope reading	...	...	...	20	22	3	4	4
			Method used					
Dry matter, %			1	1.51	1.44	2.07	2.82	4.78
Ash, % of wet weight				0.72	0.70	0.49	0.81	0.84
Ash, % of dry matter				48	48	24	29	17.6
N, % of dry matter				1.4	0.97	7.8	7.1	5.7
6.25 × N (less N present as glucosamine), % of dry matter				6.9	4.8	43	40	32
6.25 × N (less N present as glucosamine), % of organic matter				13	9.2	57	56	39
Protein by biuret, % of dry matter			2	7.0	8.5	39	29	34
Carbohydrate by orcin, % of dry matter			3	17.5	20	20	13	15
Carbohydrate by orcin after hydrolysis, % of dry matter			3	19	22	24	16	17
Ether-soluble material, % of dry matter				1.8	2.1	4.4	5.3	3.0
Cholesterol, % of dry matter			4	0.026	0.023	0.011	0.039	0.023
Reducing power (after hydrolysis) as glucose, % of dry matter			5	21	24	26	17	20
Reducing power (after hydrolysis) as glucose, % of organic matter				40	46	34	23	24
Glucosamine (after hydrolysis), % of dry matter			6	4.7	3.2	14	10.5	12.5
N-Acetylglucosamine, apparent (without hydrolysis), % of dry matter			7	Trace	Trace	0.6	0.6	0.4
Total phosphorus, % of dry matter			8	0.15	0.23	0.28	0.27	0.62

Methods used: 1, Scott Blair *et al.* (1941); 2, Mehl (1945); 3, Rimington (1940); 4, Sackett (1925); 5, Hagedorn & Jensen (1923); 6, Elson & Morgan (1933); 7, Morgan & Elson (1934); 8, King (1932).

The results show that while oestrous mucin has only 1–1.5 % dry matter, dioestrous mucin has 2–3 % dry matter and pregnancy mucin 4–5 %. The most important differences, however, are in the nitrogenous components. The mucin present in oestrus contains little nitrogen, as pointed out by Scott Blair *et al.* (1941). It contains relatively more carbohydrate and less protein and glucosamine (when calculated on an ash-free basis) than the two other types; it gives no colour with the ninhydrin reagent and a negative or feeble biuret reaction, and probably contains substances which might be classed as mucopolysaccharides (cf. Stacey, 1943). The biuret test could probably be used as a simple qualitative test for differentiating between oestrous and other cervical mucins. None of the mucins appear to contain any appreciable quantities of ethereal sulphate.

slowly. Oestrous mucin in the presence of 10 vol. of water may dissolve or swell to fill the total volume in 1 or 2 days at 15°. Mucin from pregnant cows or cows in dioestrus remains discrete in water for many days. It is remarkable how little bacterial growth occurs when mucin is allowed to stand either alone or in water at room temperature, and it is probable that it has antibacterial properties (cf. Goldsworthy & Florey, 1930). The dissolving power of a large number of reagents has been tested by measuring the time required for the discrete mass of mucin to disappear when 0.25–0.5 ml. of material was placed in 10 vol. of solution at room temperature.

Alkali. All three types of mucin dissolved in 0.1 N-NaOH within 1 hr. Solution occurred within 1 day in 0.1 N-Na₂CO₃ or 0.1 N-NH₄OH, but was not accelerated by 0.1 M-Na₃BO₄ (adjusted to pH 9.2) or 0.1 M-Na₂HPO₄ (adjusted to pH 8.0).

Acid. Dilute acetic acid or HCl (0.1 N) gave a clot with all types of mucin, but solution occurred in concentrated mineral acid.

Detergents and denaturing agents. No samples of mucin appeared to dissolve in neutral solutions of sodium dodecylsulphate (1.0 or 0.1 %), cetyltrimethylammonium bromide (1.0 or 0.1 %), urea (20 or 1 %) or guanidine chloride (20 or 1 %). The denaturing agents sometimes converted the mucin into stiffer and clearer gels than the original samples.

Enzymes. All types of cervical mucin dissolved in a 1 % solution of pancreatin (British Drug Houses Ltd.), although pregnancy mucin required 2 days for solution. Oestrous mucin alone dissolved readily in takadiastase (0.2 % of a Parke Davis preparation). No acceleration occurred with hyaluronidase (1 % suspension of dried bull testes).

Reducing agents. Inorganic reducing agents such as $\text{Na}_2\text{S}_2\text{O}_4$ (1 %), NaHSO_3 (1 %), Na_2SO_3 (1 %), $\text{Na}_2\text{S}_2\text{O}_5$ (1 %), hydroxylamine hydrochloride (1 %), or hydrazine sulphate (1 %) and solutions of sodium ascorbate (0.5 %) did not dissolve any of the types of mucin studied. But when CuSO_4 (final concentration 0.01 M) was added together with sodium ascorbate oestrous mucin dissolved in the course of an hour, but neither dioestrous nor pregnancy mucins dissolved with this reagent, which rapidly decreases the viscosity of hyaluronic acid (Hale, 1944). When H_2O_2 (final concentration 0.2 M) was also present then all types of mucin dissolved, but solution occurred just as readily if the ascorbate were omitted.

Oxidizing agents. Very mild oxidizing agents such as methylene blue (0.1 %), CuSO_4 (0.04 M), K_2CrO_7 (0.1 N), iodine (0.1 N in KI), and chloramine T (1 %) did not dissolve any type of mucin. The coloured reagents appeared to diffuse into the mucin very slowly. Iodine appeared to penetrate the mucin at about 1 cm./day; methylene blue diffused much more slowly, possibly owing to combination with the outside layer.

Solutions of H_2O_2 (0.2 M) dissolve oestrous mucin in 3 hr., dioestrous mucin in 1 day and pregnancy mucin in 2 days. The solvent action is accelerated by metals which are known to catalyze peroxide oxidations, i.e. FeSO_4 (0.01 M), CoCl_2 (0.01 M), CuSO_4 (0.01 M), and MnSO_4 (0.01 M). Iron salts were the most effective catalysts, but a precipitate was formed when they were used. Slow solution also occurred with solutions of *per* salts (e.g. $\text{K}_2\text{S}_2\text{O}_8$ (1 %) and $\text{K}_2\text{C}_2\text{O}_8$ (1 %)), and this reaction was accelerated by FeSO_4 . More rapid solution was obtained with KMnO_4 (0.1 N) but the reaction led to the formation of a dark precipitate. No solvent action was shown by KClO_3 , KClO_4 or KIO_3 (1 % solutions).

Hypochlorites and hypobromites were the most satisfactory solvents for all types of mucin. Solu-

tions of commercial NaOCl diluted to contain 1 % of available chlorine dissolved all types of mucin. Solution occurred more slowly when a solution containing 0.25 % of available chlorine was used, but not in more dilute solutions (e.g. with 0.1 % available chlorine).

Samples of mucin were placed in 10 vol. of solutions of NaOCl containing 0.5 % of available chlorine at different pH values between 7 and 11. In some experiments the solutions were acidified with acetic acid and not buffered, while in others boric acid was used to acidify and buffer the solutions. Borate did not interfere with the solvent action, which appeared to be most rapid between pH 8 and 8.5. At pH 8.5 with 0.5 % available chlorine the rate of solution appeared to be independent of the ratio of volumes of NaOCl solution and mucin, between ratios of 1:1 and 1:20, when they were allowed to interact in test-tubes. Increase in volume of NaOCl solution did not substantially increase the interfacial area in the conditions of these experiments.

Suggested irrigation fluid

The most satisfactory solution for practical use would be a solution of NaOCl containing 1 % of available chlorine and 0.1 M- HBO_3 (approx.) giving a solution of pH 8.5. Such a solution can be prepared by diluting 1 vol. of commercial NaOCl (10 % available chlorine) with 9 vol. of 0.1 M- HBO_3 (0.63 %).

The uteri of two cows were each irrigated with 250 ml. of this solution by Mr F. L. M. Dawson. The treatment had no apparent ill effects, and one of the cows was served and became pregnant 4 weeks later. Sodium hypochlorite is an effective antiseptic as well as a solvent for mucin. *Trichomonas foetus* is very susceptible to hypochlorite, being apparently killed immediately *in vitro* by a solution containing 0.001 % available chlorine. The suggested solution might therefore be effective as an irrigation fluid for the treatment of bovine trichomoniasis.

SUMMARY

1. The cervical mucin present in cows in oestrus appears to be mainly carbohydrate and may be a mucopolysaccharide, while the mucins of pregnant cows and cows in dioestrus contain both polysaccharide and protein.
2. None of the three types of mucin is attacked by hyaluronidase. They are all dissolved by neutral oxidizing agents and particularly by sodium hypochlorite.
3. A solution of sodium hypochlorite containing 1 % available chlorine and buffered with borate might be of value as an irrigation fluid in the treatment of bovine trichomoniasis.

REFERENCES

- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
 Goldsworthy, N. E. & Florey, H. (1930). *Brit. J. exp. Path.* **11**, 192.
 Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
 Hale, C. W. (1944). *Biochem. J.* **38**, 362.
 King, E. J. (1932). *Biochem. J.* **26**, 292.
 Mehl, J. W. (1945). *J. biol. Chem.* **157**, 173.
 Morgan, W. T. J. & Elson, L. A. (1934). *Biochem. J.* **28**, 988.
 Rimington, C. (1940). *Biochem. J.* **34**, 931.
 Sackett, G. E. (1925). *J. biol. Chem.* **64**, 203.
 Scott Blair, G. W., Cowie, A. T. & Folley, S. J. (1942). *J. Physiol.* **101**, 11 P.
 Scott Blair, G. W., Folley, S. J., Malpress, F. H. & Coppen, F. M. V. (1941). *Biochem. J.* **35**, 1039.
 Stacey, M. (1943). *Chem. & Ind.* **62**, 110.
 Woodman, H. E. & Hammond, J. (1925). *J. agric. Sci.* **15**, 107.

Studies in Penicillin Production by *Penicillium notatum*; in Surface Culture

1. A PRELIMINARY STUDY OF THE METABOLISM OF CARBON, NITROGEN, SULPHUR AND PHOSPHORUS

By D. J. D. HOCKENHULL, *Imperial Chemical Industries Ltd., Research Laboratories, Blackley, Manchester*

(Received 16 January 1946)

The following studies on *Penicillium notatum* were intended to prepare the way for a more detailed investigation of the method by which the mould produces penicillin, and furthermore to ascertain to what extent the production of penicillin could be increased by controlling the cultural environment.

It is important to note that the production of penicillin only represents a minute fraction of the total metabolic activity of the mould, in contrast to the production of citric acid by *Aspergillus niger*. Moreover, none of the common intermediate products of metabolism such as oxalic, fumaric and gluconic acids or glycerol, etc., could be detected in other than negligible quantities during the growth of *Penicillium notatum* under conditions favouring penicillin production. It thus appeared that an essential preliminary to investigations of the mechanism of penicillin production was to ascertain the fate of individual constituents of the culture medium. In the present investigation an attempt has been made to follow the changes undergone by some of the principal nutrient substances during the growth of the mould.

Existing methods of analysis have been modified to deal with the substances investigated.

EXPERIMENTAL

Cultural methods and materials

Composition of medium. The earlier part of the work was carried out using a modified Czapek-

Turley medium subsequently referred to as *casein medium* of which the composition was:

	%
Glucose (first grade commercial quality)	3
NaNO ₃ (analytical reagent quality)	0.3
KH ₂ PO ₄ (analytical reagent quality)	0.1
KCl (analytical reagent quality)	0.05
MgSO ₄ .7H ₂ O (analytical reagent quality)	0.025
FeSO ₄ .7H ₂ O (analytical reagent quality)	0.001
Casein, as 20% (v/v) HCl digest	0.5

This medium was made up in tap water and adjusted to pH 4.5 with 40% NaOH before sterilization.

It was communicated to us by Dr Coghill of Peoria, U.S.A., that penicillin production was greatly increased by the use of corn-steep liquor (30% total solids) in the medium. Accordingly a medium, known as *CSL medium* was adopted for commercial production of penicillin. This medium was consequently used in the investigation. Its composition was:

	%
Lactose B.P.	3
Glucose, commercial (as above)	1
Filtered corn-steep liquor	8
NaNO ₃ (analytical reagent quality)	0.3
KH ₂ PO ₄ (analytical reagent quality)	0.1
MgSO ₄ .7H ₂ O (analytical reagent quality)	0.025
ZnSO ₄ .7H ₂ O (analytical reagent quality)	0.001
MnSO ₄ .4H ₂ O (analytical reagent quality)	0.001
CuSO ₄ .5H ₂ O (analytical reagent quality)	0.001
FeSO ₄ .7H ₂ O (analytical reagent quality)	0.001

This medium was made up in tap water and adjusted to pH 4.5 with 40% (w/v) NaOH before sterilization.