cells and medium, but the previous search for potassium-binding substances has not proved successful (Roberts *et al.* 1949; Steinbach, 1950). The observations reported in this paper strongly support the view that *Alcaligenes faecalis* contains a potassium-binding constituent, but no statements can as yet be made about the nature of the potassium-binding reaction. Further work on disrupted cells aiming at the isolation and study of the potassium-binding material is required.

A potassium-binding material would account for the concentration gradients under anaerobic conditions and in the absence of oxidizable substrate. Superimposed upon that 'passive' transport of potassium is a mechanism of active transport which increases the concentration gradients in respiring cells.

SUMMARY

1. Cells of *Alcaligenes faecalis* lost most of their intracellular potassium when washed with distilled water. This treatment also removes most of the capacity to respire.

2. Respiration is restored by the addition of potassium (and an oxidizable substrate) to the suspending medium. The restoration is accompanied by an uptake of potassium against a concentration gradient.

3. The distribution of potassium between cells and medium was studied under a variety of conditions. Substantial amounts of potassium (up to 1.8 mg./ml. of cells) are accumulated in the cells against concentration gradients. Accumulation also occurs anaerobically in the absence of substrates but the final gradients reached are lower than in respiring cells.

4. Measurements of the simultaneous move-

ments of potassium, sodium and chloride show that the uptake of potassium is not accompanied by an equivalent movement of chloride or release of sodium. How electroneutrality is maintained when potassium ions move has not been established.

5. The distribution of radioactivity between cells and medium after addition of ^{42}K to the medium shows that the intracellular potassium exchanges readily with the K^+ ions of the medium. The exchange was slightly more rapid aerobically than anaerobically.

6. It is concluded that the organism contains a potassium-binding substance which behaves similarly to a colloid electrolyte or an ion-exchange resin.

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16 α -Hydroxyoestrone in the Urine of Pregnant Women

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Watson & Marrian (1955) detected the presence in extracts of the urine of pregnant women of a ketonic Kober chromogen which was more 'polar' than oestrone, and, on solvent-partition evidence, concluded that it was 16-oxo-oestradiol-17 β (16-oxo-oestra-1:3:5-triene-3:17 β -diol). More recently Marrian, Watson & Panattoni (1957) concentrated this Kober chromogen (KC-5) and showed conclusively that the major component in it must have been either 16 α -hydroxyoestrone (17-

oxo-oestra-1:3:5-triene-3:16 α -diol) or 16 β -hydroxyoestrone (17-oxo-oestra-1:3:5-triene-3:16 β -diol), and not 16-oxo-oestradiol-17 β . On biogenetic grounds the view was favoured that the major component in KC-5 was 16 α -hydroxyoestrone rather than 16 β -hydroxyoestrone, but since reduction with sodium borohydride yielded a mixture of oestriol (oestra-1:3:5-triene-3:16 α :17 β -triol) and 16-epioestriol (oestra-1:3:5-triene-3:16 β :17 β -triol) it was suggested that either 16 β -hydroxyoestrone or arti-

factually produced 16-oxo-oestradiol-17 β might also have been present in the isolated material.

For the further investigation of the nature of KC-5 it was clearly necessary to isolate a larger amount of more rigorously purified material from urine, and, since it seemed probable that the major component in the Kober chromogen might be 16 α -hydroxyoestrone, it was also decided to attempt to prepare the latter by partial synthesis.

RESULTS

Preparation and properties of 16 α -hydroxyoestrone

16 α -Hydroxyoestrone diacetate, m.p. 172–174.5°, was prepared from oestrone by the method of Leeds, Fukushima & Gallagher (1954). These authors recorded m.p. 179–180° for their preparation. However, it would appear that the latter higher melting point is that of a different polymorphic modification of the same substance, since a sample of the preparation of Leeds *et al.* (1954) kindly supplied to us by Dr Gallagher was found to melt at 168.5–172°, and this melting point was not depressed after admixture with our own preparation. Moreover, Dr Gallagher has informed us that he has also observed a lower-melting modification of the substance.

After reduction of the diacetate by sodium borohydride, followed by alkaline hydrolysis, products were obtained which, as judged by partition-chromatographic analysis used in conjunction with the Kober reaction, appeared to contain about 81–85% of oestriol and about 15–19% of 16-*epi*-oestriol. It seems unlikely that the formation of this small proportion of a 16-*epi*-oestriol-like product

could have been due to the presence in the starting material of 16 β -hydroxyoestrone diacetate or of 16-oxo-oestradiol-17 β diacetate, both of which could be expected to yield mainly 16-*epi*-oestriol on reduction by sodium borohydride followed by hydrolysis, since about the same relative amounts of the two products were formed irrespective of the number of times that the starting material had been recrystallized (Table 1). The possibility that 16-*epi*-oestriol was formed as a result of rearrangement of 16 α -hydroxyoestrone diacetate to 16-oxo-oestradiol-17 β diacetate during the reduction has not been excluded, but since the relative amounts of the two products could not be significantly altered by varying either the duration of the reaction or the amount of sodium borohydride used (Table 1) this explanation seems an unlikely one. A more probable explanation would seem to be that reduction of the 17-oxo group in 16 α -hydroxyoestrone diacetate is less stereochemically specific for the formation of a 17 β -hydroxyl group than it is in oestrone (cf. Biel, 1951), and that 17-*epi*-oestriol (oestra-1:3:5-triene-3:16 α :17 α -triol) (Prelog, Ruzicka & Wieland, 1945), which is not clearly distinguishable from 16-*epi*-oestriol in the solvent systems employed, was the 16-*epi*-oestriol-like product which was formed.

Hydrolysis of 16 α -hydroxyoestrone diacetate was effected somewhat as described by Leeds *et al.* (1954) for the rearrangement and hydrolysis of 16 α :17 α -epoxyandrostane-3 β :17 β -diol diacetate to 17-oxoandrostane-3 β :16 α -diol. After recrystallization the product melted at 238–240.5°, with marked shrinkage at about 216°, and had $[\alpha]_D^{16} + 180^\circ$ (ethanol). Acetylation of the hydrolysis product

Table 1. *Reduction of 16 α -hydroxyoestrone diacetate by sodium borohydride in methanolic solution: relative proportions of oestriol and '16-*epi*-oestriol' in the products after alkaline hydrolysis*

Wt. of 16 α -hydroxy- oestrone diacetate (mg.)	Previous recrystallizations	Wt. of NaBH ₄ (mg.)	Reduction time (min.)	Composition of hydrolysed reduction product (%)	
				Oestriol	'16- <i>epi</i> - Oestriol'
20.3	2 $\times$ ethyl acetate- <i>n</i> -hexane	15 + 10	40 + 60	81.2	18.8
20.0	2 $\times$ ethyl acetate- <i>n</i> -hexane 1 $\times$ benzene- <i>n</i> -hexane 3 $\times$ methanol	15 + 10	40 + 60	81.5	18.5
21.0	2 $\times$ ethyl acetate- <i>n</i> -hexane 1 $\times$ benzene- <i>n</i> -hexane 3 $\times$ methanol	5 + 2	15 + 20	83.5	16.5
20.0	2 $\times$ ethyl acetate- <i>n</i> -hexane 2 $\times$ methanol	4 + 2.5	15 + 20	81.2	18.8
20.0	2 $\times$ ethyl acetate- <i>n</i> -hexane 2 $\times$ methanol	2.5	35	82.2	17.8
20.0	2 $\times$ ethyl acetate- <i>n</i> -hexane 1 $\times$ benzene- <i>n</i> -hexane 5 $\times$ methanol	4 + 2	15 + 20	85.3	14.7
5.3	2 $\times$ ethyl acetate- <i>n</i> -hexane 2 $\times$ methanol	5	40	81.8	18.2

yielded an acetate, m.p. 169–173°, $[\alpha]_D^{20} + 149$ (ethanol), and on admixture with 16 α -hydroxy-oestrone diacetate the melting point was not depressed. It was evident, therefore, that the hydrolysis product of the diacetate was indeed 16 α -hydroxyoestrone.

16 α -Hydroxyoestrone was also prepared more conveniently by treatment of 16 α :17 α -epoxyoestra-1:3:5-triene-3:17 β -diol diacetate with methanolic sulphuric acid at room temperature, when rearrangement and hydrolysis occurred (cf. Leeds *et al.* 1954).

The melting-point behaviour of 16 α -hydroxy-oestrone requires special comment. The melting point, like that of the crude KC-5 isolated by Marrian *et al.* (1957), was not depressed after admixture with 16-oxo-oestradiol-17 β (m.p. 239–241.5°), and this fact suggested that at or near its melting point 16 α -hydroxyoestrone might undergo rearrangement to 16-oxo-oestradiol-17 β . On re-determining the melting point of 16 α -hydroxy-oestrone with a more rapid rate of heating over the range 205–230° it was seen that the shrinkage previously observed at about 216° was in fact a fairly sharp melt, followed immediately by resolidification which was complete at about 222°. This suggested that the lower melting point might be the true one of 16 α -hydroxyoestrone and that the higher one might be that of the hypothetical rearrangement product. In fact 16 α -hydroxyoestrone was found to yield, when heated at 220–230° *in vacuo*, 16-oxo-oestradiol-17 β , which was identified by its physical properties and those of its diacetate.

Reduction of 16 α -hydroxyoestrone by sodium borohydride yielded a mixture containing about 90 and 10% respectively of oestriol-like and 16-*epi*-oestriol-like products. Accordingly, as with the diacetate, it would appear either that some rearrangement occurred during the reaction or that a small proportion of 17-*epi*oestriol was formed. As catalytic reduction with hydrogen and platinum oxide in neutral ethanolic solution yielded a mixture which contained a somewhat higher proportion (30%) of the '16-*epi*oestriol-like' product than was found after sodium borohydride reduction, the latter explanation seems the more probable one, since under these conditions it is unlikely that any rearrangement of 16 α -hydroxyoestrone to 16-oxo-oestradiol-17 β would have occurred.

On reduction with sodium borohydride the KC-5 isolated from urine by Marrian *et al.* (1957) yielded a relatively high proportion (45%) of 16-*epi*-oestriol, which was identified as such with certainty; and in explanation of this finding the possibility was considered, among others, that the isolated material might have consisted of a mixture of 16 α -hydroxy-oestrone and 16-oxo-oestradiol-17 β produced from the former by rearrangement during the extraction

of the urinary phenolic fraction with aqueous sodium hydroxide. The fact that the urinary product had a significantly lower dextrorotation than that of 16 α -hydroxyoestrone was clearly consistent with this possibility, and to investigate the latter further the behaviour of 16 α -hydroxyoestrone in aqueous sodium hydroxide was studied.

In the preparation of the phenolic fraction of a urinary extract the time during which the fraction remains in alkaline solution is about 5–10 min.; accordingly, in the first instance, the effect on the optical rotation of 16 α -hydroxyoestrone of allowing it to stand in aqueous *N*-sodium hydroxide for 10 min. at room temperature was examined. The phenolic product isolated from the reaction mixture showed a decrease in dextrorotation compared with that of the starting material which would have corresponded to the rearrangement of about 20% of the latter to 16-oxo-oestradiol-17 β . That the observed decrease in dextrorotation was indeed due to partial rearrangement to 16-oxo-oestradiol-17 β was shown by a second experiment in which 16 α -hydroxyoestrone was treated with aqueous sodium hydroxide for 2 hr.: the product was identified as 16-oxo-oestradiol-17 β by its physical properties and those of its diacetate. These findings support the view that the KC-5 isolated from pregnancy urine by Marrian *et al.* (1957) was a mixture of 16 α -hydroxy-oestrone and artifactually produced 16-oxo-oestradiol-17 β .

Isolation, purification and identification of KC-5

By use of the procedures described by Marrian *et al.* (1957) crude KC-5, $[\alpha]_D^{18} + 114^\circ$ (ethanol), was obtained from enzymically hydrolysed late-pregnancy urine. An attempt to purify directly the dextrorotatory component in this product by recrystallization from methanol failed, and accordingly it was decided to attempt purification via the acetate.

The acetylated product yielded two crops of apparently pure crystalline material: (a) m.p. 172.5–174.5°, $[\alpha]_D^{20} + 146^\circ$ (ethanol); and (b) m.p. 170–173°, $[\alpha]_D^{18} + 157^\circ$ (ethanol). Analysis of (a) gave C and H values in good agreement with those required for a compound of the formula $C_{22}H_{26}O_5$.

Conclusive proof of the identity of KC-5 acetate and 16 α -hydroxyoestrone diacetate was provided as follows: (i) The melting point of product (b) was not depressed after admixture with 16 α -hydroxy-oestrone diacetate. (ii) The absorption spectra of solutions of KC-5 acetate (b) and 16 α -hydroxy-oestrone diacetate in concentrated sulphuric acid over the range 220–550 $m\mu$. after 2 hr. at 25° were identical (Fig. 1). (iii) The infrared spectra of KC-5 acetate (b) and 16 α -hydroxyoestrone diacetate, which were determined in potassium chloride disks by Dr R. K. Callow of the National Institute

Fig. 1. Absorption spectra of H_2SO_4 solutions of (A) 16 α -hydroxyoestrone diacetate and (B) KC-5 acetate after 2 hr. at 25°.

for Medical Research, 'showed complete similarity in all features, both in the main bands . . . and in all the bands in the "fingerprint" region . . . '.

Hydrolysis of KC-5 acetate with methanolic sulphuric acid at room temperature yielded a product which had $[\alpha]_D^{18} + 169^\circ$ (ethanol) and m.p. about 215° and 237.5–240°. The absorption spectrum of a solution of the product in concentrated sulphuric acid determined after 2 hr. closely resembled, but was not identical with, that of a similarly prepared solution of 16 α -hydroxyoestrone. The slight differences between the absorption spectra and the fact that the optical rotation of the product was slightly low suggested that during the hydrolysis of KC-5 diacetate slight rearrangement had occurred. Unfortunately there was insufficient of the hydrolysis product to permit of more rigorous purification.

It is considered that completely satisfactory proof for the identity of KC-5 and 16 α -hydroxyoestrone has been obtained.

EXPERIMENTAL

Methods

The procedures used for partition-chromatographic analysis, for the Kober reaction and for the determination of melting points, were as described by Marrian *et al.* (1957). All melting points are uncorrected for emergent stem.

Preparation and properties of 16 α -hydroxyoestrone

16 α -Hydroxyoestrone diacetate. This was prepared from 16 α :17 α -epoxyoestra-1:3:5-triene-3:17 β -diol diacetate by treatment with perchloric and acetic acids as described by Leeds *et al.* (1954). The crude product of rearrangement was purified by filtration in benzene solution through a column of deactivated [10% (w/v) of water] Al_2O_3 and then by two

crystallizations from ethyl acetate-*n*-hexane. The material had m.p. 169–172.5°, and partition-chromatographic analysis in the system 90% (v/v) ethanol in water/*n*-hexane did not reveal the presence of more than one Kober chromogen. Different portions of this material were further recrystallized from methanol and benzene-*n*-hexane. The portion for analysis was further recrystallized once from benzene-*n*-hexane and thrice from methanol, when it had m.p. 172–174.5°, $[\alpha]_D^{18} + 126^\circ$ (c, 0.502 in chloroform), $[\alpha]_D^{19} + 153^\circ$ (c, 0.503 in ethanol) (Found: C, 71.2; H, 6.8. Calc. for $\text{C}_{22}\text{H}_{30}\text{O}_5$: C, 71.3; H, 7.1%).

Reduction of 16 α -hydroxyoestrone diacetate by sodium borohydride. The reduction of 16 α -hydroxyoestrone diacetate by Na borohydride was studied in a series of experiments in which samples of the diacetate which had been recrystallized in different ways were allowed to react at room temperature in methanolic solution with varying amounts of the reductant for varying times. After hot alkaline hydrolysis the reaction mixtures were diluted with water, acidified with HCl and extracted with ether. The ether extracts were washed with 8.5% (w/v) NaHCO_3 and with water and evaporated to dryness. About 50 μg . of each product was analysed by partition chromatography, with the system 70% (v/v) methanol in water-ethylene dichloride, and in each case the presence of two different Kober chromogens chromatographically indistinguishable from oestriol and 16-epioestriol respectively was detected. The more 'polar' Kober chromogen was isolated in one experiment and identified as oestriol by a mixed m.p. with an authentic specimen of the latter; in another experiment it was identified after acetylation by a mixed m.p. with authentic oestriol triacetate. Owing to lack of material conclusive evidence concerning the nature of the less 'polar' Kober chromogen was not obtained. The results of these experiments are summarized in Table 1.

Hydrolysis of 16 α -hydroxyoestrone diacetate. To a solution of 170 mg. of 16 α -hydroxyoestrone diacetate [m.p. 172–174.5°; $[\alpha]_D^{18} + 150^\circ$ (ethanol)] in 20 ml. of methanol was added 5 ml. of 5*N*- H_2SO_4 and the mixture allowed to stand at room temperature for 5 days. After dilution with 140 ml. of ethyl acetate the solution was washed with 8.5% (w/v) NaHCO_3 and water, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The product (130 mg.) on crystallization from methanol yielded 52 mg. of colourless crystals, m.p. about 216° and 238–240.5°, $[\alpha]_D^{18} + 180^\circ$ (c, 0.490 in ethanol) (Found: C, 75.6; H, 7.3. Calc. for $\text{C}_{18}\text{H}_{22}\text{O}_3$: C, 75.5; H, 7.7%).

Acetylation of a portion of this material with acetic anhydride and pyridine at room temperature overnight yielded a product which after crystallization from methanol had m.p. 169–173° and $[\alpha]_D^{20} + 149^\circ$ (c, 0.504 in ethanol). The melting point was not depressed after admixture either with the above-described 16 α -hydroxyoestrone diacetate (m.p. 172–174.5°) or a sample of the latter (m.p. 168.5–172°) supplied by Dr T. F. Gallagher.

16 α -Hydroxyoestrone from 16 α :17 α -epoxyoestra-1:3:5-triene-3:17 β -diol diacetate. The epoxyacetate (45 mg.) was dissolved in a mixture of 4 ml. of methanol and 1 ml. of 5*N*- H_2SO_4 , and the solution allowed to stand at room temperature for 5 days. After dilution with ethyl acetate the solution was washed with 85% (w/v) NaHCO_3 and water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The product (37 mg.) yielded 11 mg. of colourless needles after crystallization from methanol. These had m.p.

237–239° (shrinkage at 215° not recorded as a melt) and $[\alpha]_D^{25} + 174^\circ$ (c, 0.500 in ethanol). Acetylation at room temperature with acetic anhydride and pyridine yielded a product which had m.p. 167–172° after one crystallization from methanol. The melting point was not depressed after admixture with 16 α -hydroxyoestrone diacetate (m.p. 172–174.5°).

Reduction of 16 α -hydroxyoestrone by sodium borohydride. To a solution of 5.0 mg. of 16 α -hydroxyoestrone in 2.5 ml. of methanol was added 5 mg. of Na borohydride. After standing at room temperature for 40 min. the solution was diluted with water, acidified with HCl and extracted with ether. The ether extract, after washing with NaHCO₃ and with water, was evaporated to dryness. Analysis by partition chromatography [70% (v/v) methanol in water–ethylene dichloride] of 50 μ g. of the product showed the presence of 90 and 10% respectively of 'oestriol-like' and '16-epioestriol-like' Kober chromogens.

Catalytic reduction of 16 α -hydroxyoestrone. A solution of 155 μ g. of 16 α -hydroxyoestrone in 4 ml. of ethanol was shaken for 30 min. at atmospheric pressure with about 0.4 mg. of Adams platinum oxide catalyst in an atmosphere of hydrogen. After filtration the solution was evaporated to dryness, and a Girard separation with trimethylammonium-acetohydrazide chloride carried out on a portion of the product to remove any unreduced ketonic material. Analysis of the non-ketonic fraction by partition chromatography [70% (v/v) methanol in water–ethylene dichloride] showed the presence of 70 and 30% respectively of 'oestriol-like' and '16-epioestriol-like' Kober chromogens.

Rearrangement of 16 α -hydroxyoestrone to 16-oxo-oestradiol-17 β by heat. 16 α -Hydroxyoestrone ($[\alpha]_D^{18} + 170^\circ$ (ethanol); 25 mg.) was introduced into a tube, which was then sealed under reduced pressure (1.4×10^{-4} mm. Hg). The tube was immersed in an oil bath which was rapidly heated to 220°, maintained at this temperature for 5 min., and finally heated to 230° for a further 2 min. After cooling, the slightly yellow semicrystalline product was washed several times with *n*-hexane and then crystallized once from methanol. The crystallized product melted at 236–238° with only very slight shrinkage at 217°, and had $[\alpha]_D^{18} - 69^\circ$ (c, 0.508 in ethanol). Acetylation at room temperature with acetic anhydride and pyridine yielded a product with m.p. 133–137°, and the m.p. after admixture with authentic 16-oxo-oestradiol-17 β diacetate (m.p. 134–137°) was 133–136.5°.

Rearrangement of 16 α -hydroxyoestrone to 16-oxo-oestradiol-17 β in alkaline aqueous solution. To a solution of 10.4 mg. of 16 α -hydroxyoestrone ($[\alpha]_D^{17} + 181^\circ$ (ethanol)) in 1 ml. ethanol was added 100 ml. of *N*-NaOH. After standing for 10 min. at room temperature sufficient 5*N*-H₂SO₄ was added to neutralize nine-tenths of the NaOH and neutralization (to pH 9) then completed by passing in a stream of CO₂. The reaction mixture was extracted with ether and the ether extract, after washing with 2% (w/v) NaHCO₃ and water and drying over anhydrous Na₂SO₄, was evaporated to dryness. The unpurified product showed $[\alpha]_D^{17} + 122^\circ$ (c, 0.50 in ethanol).

To investigate the possibility that the fall in optical rotation observed in this experiment had been due to the rearrangement of 16 α -hydroxyoestrone to 16-oxo-oestradiol-17 β , the following further experiment was carried out: To a solution of 48 mg. of 16 α -hydroxyoestrone ($[\alpha]_D^{17} + 181^\circ$ (ethanol)) in 2 ml. of ethanol was added 100 ml. of *N*-NaOH. The solution was allowed to stand at room temperature for 2 hr. and then acidified with 5*N*-H₂SO₄. The reaction mixture was extracted with ether, and the extract, after

washing with 5% (w/v) NaHCO₃ and water, dried over anhydrous Na₂SO₄ and evaporated to dryness. The crude product (39.5 mg.) had $[\alpha]_D^{20} - 83^\circ$ (c, 0.493 in ethanol). After crystallization once from methanol 19 mg. of material having m.p. 236.5–238.5° and $[\alpha]_D^{19} - 95^\circ$ (c, 0.492 in ethanol) was obtained. Acetylation of this in the usual way yielded a product which, after crystallization twice from ethyl acetate–*n*-hexane, had m.p. 133–136°, and the m.p. after admixture with authentic 16-oxo-oestradiol-17 β diacetate was 133–137°.

Identification of KC-5 from pregnancy urine as 16 α -hydroxyoestrone

Isolation of crude KC-5. By exactly the same methods as previously described by Marrian *et al.* (1957) a fraction weighing 125 mg. and containing 94 mg. of KC-5 (calc. as oestriol) was obtained by partition chromatography from the ether-soluble ketonic-phenolic fraction of 256 l. of enzymically-hydrolysed pooled late-pregnancy urine. Pigmented and gummy impurities were removed from this material by two washings with small volumes of methanol at –20°, and thus were obtained 64 mg. of a nearly white solid having $[\alpha]_D^{18} + 114^\circ$ (c, 0.495 in ethanol). After two crystallizations from methanol 5.9 mg. of material having $[\alpha]_D^{20} - 41^\circ$ (c, 0.523 in ethanol) were obtained. In view of this failure to concentrate the dextrorotatory component in the mixture directly all the fractions containing KC-5 were recombined, and then washed once with a small volume of methanol at –20° to remove pigment and gum.

Isolation of 16 α -hydroxyoestrone diacetate from acetylated crude KC-5. The crude KC-5 (63 mg.) obtained as described above was treated with 2 ml. of acetic anhydride and 2 ml. of pyridine overnight at room temperature. One crystallization from ethyl acetate–*n*-hexane and three from methanol yielded 15.7 mg. of a product (a) which had m.p. 172.5–174.5° and $[\alpha]_D^{20} + 146^\circ$ (c, 0.529 in ethanol) (Found: C, 71.3; H, 6.9. Calc. for C₂₂H₃₆O₅: C, 71.3; H, 7.1%).

From the material obtained after combining the mother liquors of the crystallizations described above a further batch (b) of 18.7 mg. of material was obtained by washing once with a small volume of methanol at –20° and crystallizing once from ethyl acetate–*n*-hexane. This had m.p. 170–173° and $[\alpha]_D^{18} + 157^\circ$ (c, 0.512 in ethanol). The mixed m.p. with authentic 16 α -hydroxyoestrone diacetate was 170–173°.

Hydrolysis of KC-5 acetate. To a solution of 19.6 mg. of a mixture of batches (a) and (b) of the purified acetate in 4 ml. of methanol was added 0.5 ml. of 5*N*-H₂SO₄ and the mixture allowed to stand at room temperature for 5 days. After dilution with ethyl acetate the reaction mixture was washed with 8.5% (w/v) NaHCO₃ and water, dried over anhydrous Na₂SO₄ and evaporated to dryness. The product was crystallized once from a small volume of methanol at –20°, yielding 11.5 mg. of material having m.p. about 215° and 237.5–240° and $[\alpha]_D^{18} + 169^\circ$ (c, 0.501 in ethanol). The mixed m.p. with authentic 16 α -hydroxyoestrone (m.p. about 216° and 238–240.5°) was about 217° and 238–240.5°.

DISCUSSION

The results reported here have conclusively shown the presence of 16 α -hydroxyoestrone in extracts of enzymically hydrolysed late-pregnancy urine: and

in view of the finding that this compound readily undergoes rearrangement in alkaline solution to 16-oxo-oestradiol-17 β there can be little doubt that the KC-5 previously isolated by Marrian *et al.* (1957) was indeed a mixture of 16 α -hydroxyoestrone and artifactually produced 16-oxo-oestradiol-17 β as was suggested. The available evidence strongly suggests that extracts of late-pregnancy urine contain no 16-oxo-oestradiol-17 β other than that which must be artifactually formed from 16 α -hydroxyoestrone during the extraction of the phenolic fraction by aqueous alkali from ether. In view of this the recent claim by Levitz, Spitzer & Twombly (1956) to have detected the presence of radioactive 16-oxo-oestradiol-17 β in the urine of human subjects after the administration of [16-¹⁴C]oestradiol-17 β must be treated with reserve.

The isolation of 16 α -hydroxyoestrone from urine lends support to the previous suggestion of Marrian *et al.* (1957) that this compound may be formed from oestrone by 16 α -hydroxylation and may be the metabolic intermediate in the 'hydration' of oestrone to oestriol.

SUMMARY

1. 16 α -Hydroxyoestrone diacetate, prepared from 16 α :17 α -epoxyoestra-1:3:5-triene-3:17 β -diol diacetate by the method of Leeds *et al.* (1954), yielded 16 α -hydroxyoestrone on cold acid hydrolysis. 16 α -Hydroxyoestrone was also prepared directly from the epoxyacetate by cold acid treatment.

2. 16 α -Hydroxyoestrone readily undergoes rearrangement to 16-oxo-oestradiol-17 β in aqueous alkali at room temperature. The same rearrangement occurs when it is heated *in vacuo* to 220–230°.

3. A preparation of the ketonic-phenolic Kober chromogen (KC-5) prepared from enzymically-

hydrolysed late-pregnancy urine as described by Marrian *et al.* (1957) yielded a product after acetylation which has been conclusively identified as 16 α -hydroxyoestrone diacetate.

4. In view of the rearrangement undergone by 16 α -hydroxyoestrone in alkaline solution it is concluded that KC-5 as isolated from urine by the methods described consists of 16 α -hydroxyoestrone contaminated with about 20% of artifactually produced 16-oxo-oestradiol-17 β .

5. It is suggested that 16 α -hydroxyoestrone may be the metabolic intermediate between oestrone and oestriol.

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Synthesis of Porphyrins by Cell Suspensions of *Tetrahymena vorax*: Effect of Members of the Vitamin B Group

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It is well established that the biosynthesis of the porphyrin nucleus in animal tissues proceeds by condensation of glycine with an unsymmetrical derivative of succinate (derived from the tricarboxylic acid cycle) with intermediate formation of

δ -aminolaevulinic acid (ALA) and porphobilinogen (Shemin, 1955; Dresel & Falk, 1956*a-c*; Falk, Dresel, Benson & Knight, 1956; Berlin, Neuberger & Scott, 1956*a, b*). There is evidence that a similar pathway occurs in photosynthetic bacteria of the Athiorhodaceae group (Lascelles, 1955; Cooper, 1956).

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