Investigations of D-Homosteroids as Potential Carcinogens

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The high carcinogenic potency of 20-methylcholanthrene and its close structural relationship to the steroids led to the hypothesis that carcinogenic hydrocarbons might arise from endogenous steroids under abnormal metabolic conditions.

In its original form this hypothesis envisaged the conversion of cholesterol or deoxycholic acid (I) via dehydronorcholene (II) into 20-methylcholanthrene (III), a process attainable by pyrolytic methods in

these are known to undergo facile conversion to derivatives of 17 α -methyl-D-homoandrostande (V) by adsorption at a moist, neutral aluminium oxide surface at 20° (Stavely, 1940; Prins & Reichstein, 1940; Shoppee & Prins, 1943). Whilst there is no evidence that the dehydrogenation of derivatives of 17 α -methyl-D-homoandrostande (V) to derivatives of 3-methylchrysene (III), accomplished *in vitro* (Ruzicka & Meldahl, 1940), occurs *in vivo* (cf. the

the laboratory (Wieland & Dane, 1933; Fieser & Newman, 1935; Rossner, 1937), under physiological conditions. It was supported by the reported carcinogenic activity of benzene extracts of *Escherichia coli* cultures grown in presence of dehydronorcholene (Druckrey, Richter & Vierthaler, 1941); this activity cannot, however, be due to some transformation product, since dehydronorcholene, which is non-carcinogenic, is unaltered by *Esch. coli* (Butenandt & Dannenberg, 1950).

An alternative form of the hypothesis is suggested by the fact that the structural analogy between a cholic acid (I) and 20-methylcholanthrene (III) is paralleled by the even closer analogy between 17 α -methyl-D-homoandrostande (V) and 3-methylchrysene (VI).

The normal activity of the adrenal cortex leads to 17 α -hydroxy-20-ketosteroids (as IV: R = H or OH);

dehydrogenation (II) $\rightarrow$ (III)), analogies may be quoted, e.g. the biological dehydrogenation of cyclohexanecarboxylic acid to benzoic acid (Bernhard & Cafisch-Weill, 1945; Dickens, 1947; Beer, Dickens & Pearson, 1949) and of (+)-oestrone to (+)-equilenin in the pregnant mare (Girard, Sandulesco, Fridenson, Gaudefroy & Rutgers, 1932; Girard, Sandulesco, Fridenson & Rutgers, 1932). Further, although chrysene is non-carcinogenic, some of its homologues are active; thus 1:2-dimethylchrysene is carcinogenic (Hewett, 1940).

There is a considerable body of evidence that the adrenal cortex may be implicated in tumorigenesis (cf. Woolley & Little, 1945), and it has been observed that adrenocortical hormone production and metabolism are very frequently disturbed in neoplastic disease (Lieberman, Dobriner, Hill, Fieser & Rhoads, 1948). If the facile change (IV $\rightarrow$ V) (cf.

Hirschmann & Hirschmann, 1947) can occur *in vivo* by adsorption at a lipoprotein surface, then in hyperfunction of the adrenal cortex *D*-homosteroids might arise in such quantity as to compel the organism to resort to abnormal metabolic paths; one path could lead to derivatives of 3-methylchrysene (VI). It seemed therefore of interest to prepare some *D*-homosteroids and some derivatives of 3-methylchrysene, and to test them for carcinogenic activity. Owing to external circumstances, the work had to be discontinued, and the present account deals with the simplest representatives, 17 α -methyl-*D*-homoandrostande and 3-methylchrysene.

17 α -Methyl-*D*-homoandrostande is not carcinogenic in stock mice when repeatedly applied to the skin in 0.5% solution in benzene. 3-Methylchrysene appears to be a weak carcinogen, for although it produced no tumours in mice by painting, by the graft technique of Dr Horning (Royal Cancer Hospital) it caused proliferation of the prostatic epithelium with active mitosis in the glandular epithelium and infiltration of the stroma, processes which precede tumour formation.

EXPERIMENTAL

Melting points were determined on a Kofler block (limit of error $\pm 2^\circ$). Solvents for chromatographic operations were rigorously purified and dried and, unless stated otherwise, aluminium oxide (Spence type H, activity II) was used. For drying of ethereal extracts, brief treatment with anhydrous sodium sulphate was used.

17 α -Methyl-*D*-homoandrostande. This was obtained by Wolff-Kishner reduction of 17 α -methyl-*D*-homoandrostand-3-one, m.p. 182° (Ruzicka & Meldahl, 1940; Shoppee & Prins, 1943). After purification by chromatography, repeated crystallization from CHCl_3 : methanol, and sublimation at 130 – $140^\circ/0.01$ mm., it had m.p. 108 – 109° .

3-Methylchrysene. This was obtained by combination of procedures due to Bachmann & Struve (1940) and Cook & Schoental (1945). α -Tetralone with amalgamated zinc and

methyl γ -bromocrotonate (Ziegler, Schumann, Winkelmann, Späth & Schaaf, 1942) gave methyl γ -1-(3:4-dihydronaphthyl)crotonate, isomerized by palladium black (prepared according to Heilbron, Sexton & Spring, 1929) at 280° for 3 hr. in an atmosphere of CO_2 , and hydrolysed by 4% ethanolic KOH to γ -1-naphthylbutyric acid, m.p. 97 – 98° . This was cyclized directly with anhydrous HF (cf. Fieser & Hershberg, 1939; Fieser & Johnson, 1940) or as the chloride with SnCl_4 (cf. Wilds, 1942) to yield 1-keto-1:2:3:4-tetrahydrophenanthrene, m.p. 93° . Repetition of this series of reactions with the last named ketone gave seriatim methyl γ -1-(3:4-dihydrophenanthryl)crotonate, γ -1-phenanthrylbutyric acid, m.p. 155 – 157° , and 3-keto-3:4:5:6-tetrahydrochrysene, m.p. 229 – 230° , undepressed by a specimen, m.p. 228 – 229° , prepared by Bachmann & Struve (1940).

The tetracyclic ketone by treatment with methyl magnesium iodide, and simultaneous dehydration and dehydrogenation of the resulting carbinol with palladized charcoal (prepared according to Newman & Zahm, 1943) for 1 hr. at 300° in an atmosphere of CO_2 , gave 3-methylchrysene; this was crystallized from benzene, further purified by filtration of a benzene solution through a column of aluminium oxide, and recrystallization from benzene, m.p. 253 – 254° , undepressed by admixture with a specimen, m.p. 250° , prepared by Bachmann & Struve (1940).

SUMMARY

The possible conversion of 17 α -hydroxy-20-ketosteroids derived from the adrenal cortex into *D*-homosteroids and so into derivatives of 3-methylchrysene is discussed. *D*-Homoandrostande is non-carcinogenic, but 3-methylchrysene, prepared by a simple route from α -tetralone, is a weak carcinogen.

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The Fluorescence of Teeth under Ultraviolet Irradiation

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The observation that teeth fluoresce when irradiated with ultraviolet light was reported by Stubel (1911). He studied a whole series of tissues and found that the teeth exhibited an intense blue fluorescence second only in brilliance to the lens of the eye. Some years later, Benedict (1928) noted that dentine fluoresced much more strongly than enamel and with a seemingly bluer light. He observed that the white spot indicative of 'beginning' dental caries did not fluoresce. He produced a similar effect by scratching through a paraffin-coated tooth and placing in dilute acetic acid overnight. Benedict also noted that ashed enamel did not fluoresce, nor did dentine which had been boiled in 50 % sodium hydroxide, whilst on decalcifying dentine with dilute nitric acid the remaining organic matrix retained its fluorescence to an appreciable extent. Tiede & Chomse (1934) were of the opinion that it was the organic protein compounds of the teeth which were responsible for the phenomenon. They said that teeth extracted with alkaline glycerol retained enough organic matter to fluoresce, but that the property was lost after treatment with hydrogen peroxide and ammonia. Glasser & Fonda (1938), on the other hand, believed that the fluorescence was linked with the mineral phase. They used ox teeth, presumably the enamel only, since they stated that their material contained 1 % protein. According to these workers the fluorescence was destroyed by burning out the organic content at 600° but was increased by heating at 400°. They found that after 'solution of the teeth in acid and precipitation with alkali' the fluorescence was restored by heating at 400°. Bodecker (1939) put forward the suggestion that dental lymph was responsible for the fluorescence, although vital and non-vital teeth both fluoresce. Hadjioloff & Russewa (1943) observed that enamel, dentine and cementum appeared blue under ultraviolet light and that enamel and dentine sometimes appeared yellow. They reported that by decalcifying with a half-

saturated solution of sodium chloride in dilute hydrochloric acid the teeth lost their ability to fluoresce.

The phenomenon of tooth fluorescence poses two main questions. First, what substance or substances are responsible and, secondly, why does the carious lesion cease to fluoresce? The answer to these two questions may be of fundamental importance in the aetiology of dental caries. If, for example, the active material should prove to be organic in nature, it would mean that disintegration of organic matter occurs at a very early stage in the carious process. The object of the present investigation was to observe the fluorescence in various tooth specimens and to explore techniques for extracting the active substances from the teeth, with a view to finding some information concerning their chemical nature.

EXPERIMENTAL

Observations on the fluorescence of teeth

A 'dark bulb' mercury-vapour lamp was used routinely as a source of ultraviolet radiation. The appearance of the fluorescence was compared with that produced by a 'Hanovia' fluorescence lamp, Model 11, with Wood's glass filter which transmits in the region of 366 m μ . No differences were detected. Prior to their examination, teeth were freed from adhering tissues, calculus and stains by careful use of brush and pumice followed by a thorough washing in water. Observations were made on whole teeth and on sections.

Sound teeth

(i) *Enamel*. Enamel normally fluoresced bluish white but occasionally tinged with yellow. Pl. 2 shows a group of three sound teeth photographed under artificial white light and under ultraviolet light; the differences in colour intensity are lost in the black and white print, but an impression can be gained of the lustre of the fluorescent light.