

It is apparent that *Digitalis purpurea* can utilize cholesterol and converts it to several metabolites. The important intermediate pregnenolone constitutes about 1% ( $2.9 \cdot 10^4$  dpm) of the total metabolites ( $2.7 \cdot 10^6$  dpm). This indicates a minimum 0.005–0.01% conversion of the administered cholesterol to pregnenolone.

Since the pregnenolone was characterized by dilution and recrystallization to constant specific activity of the free alcohol, as well as of the acetate, it was desired to provide additional evidence for its identity. With this in mind a third isolation experiment was carried out as above and the obtained pregnenolone ( $20.9 \cdot 10^3$  dpm/mM) was converted to its acetate ( $20.7 \cdot 10^3$  dpm/mM). This acetate was then submitted to Baeyer-Villiger oxidation as previously described by us<sup>11</sup>. Upon saponification of the recovered product the known  $3\beta, 5\alpha, 6\beta, 17\beta$ -tetrahydroxy- $5\alpha$ -androstane was obtained<sup>11</sup> which showed a specific activity of  $20.9 \cdot 10^3$  dpm/mM. This transformation corroborates fully the identity of the isolated metabolite.

The evidence presented tends to support the hypothesis that cholesterol or a closely related triterpene are the 'primary' sterols formed in the plant. It seems also that pregnenolone, or a related  $C_{21}$ -20-ketone may play an important role in the biosynthesis of butenolides<sup>12</sup>. In any event this constitutes proof that cholesterol may serve as a precursor in the synthesis of plant sterols<sup>13,14</sup>.

*Résumé.* Un échantillon purifié de cholestérol-4- $C^{14}$  a été absorbé par des feuilles de 2 plantes de *Digitalis purpurea*. Après 18 jours on a extrait les plantes et séparé les composés radioactifs. Environ 1% des métabolites globales a été constaté en forme de pregnénolone. Ainsi le cholestérol peut agir comme précurseur des stéroïdes végétaux.

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<sup>11</sup> E. CASPI, D. O. LEWIS, D. M. PIATAK, K. V. THIMANN, and A. WINTER, *J. Am. chem. Soc.* **87**, 3224 (1965).

<sup>12</sup> R. D. BENNETT and E. HEFTMANN, *Science* **149**, 653 (1965) have recently demonstrated the conversion of pregnenolone to progesterone in *Holarrhena floribunda*.

<sup>13</sup> DR. R. D. BENNETT and Mr. E. HEFTMAN informed us that they have also isolated pregnenolone upon administration of cholesterol to *Haplopappus heterophyllus* (Phytochemistry, in press).

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### Ceruloplasmin in Human Semen

Several enzymes have been detected in human semen: phosphatases<sup>1,2</sup>, lactic dehydrogenase<sup>3</sup>, transaminases<sup>4-6</sup> and nucleoside phosphorylase<sup>7</sup>.

The presence of ceruloplasmin was suspected by us, since this fluid contains copper<sup>8</sup> and is very rich in mucoproteins<sup>9</sup>. Adrenaline oxidase has also been detected in human prostatic gland and seminal vesicle<sup>1</sup>. As ceruloplasmin is an oxidase for this amine<sup>10</sup>, we thought it of interest to study its activity in human semen.

*Material and methods.* Semen samples from 21 men were used throughout this study and several tests were made to establish criteria of normality (sperm concentration, motility and vitality, volume of ejaculate, percentile quantity of abnormal forms). Men were classified into 3 groups: 5 normospermic (spermatozoa number between 56 and 150 millions/ml), 11 oligospermic (between 0.5 and 40 millions/ml) and 5 azospermic.

Ceruloplasmin was determined enzymatically by the method of RAVIN<sup>11</sup> and values were expressed in  $\mu\text{g}/100\text{ ml}$ .

*Results.* The results obtained are presented in the Table.

Normospermic semen showed very low values of ceruloplasmin ( $594 \pm 87 \mu\text{g}/100\text{ ml}$ ) as compared with

dog semen (average 190 mg/100 ml). In this animal, copper values are also much more elevated than in humans (average 814  $\mu\text{g}/100\text{ ml}$ )<sup>4</sup>.

A statistically significant decrease of ceruloplasmin activity was observed in azospermic samples in relation to normospermic ones: azospermic/normospermic:  $t = 3.417$ ,  $P < 0.01$ . Decrease for oligospermia was not statistically significant:  $t = 0.415$ ,  $P > 0.5$ .

*Résumé.* La céruloplasmine a été déterminée dans le liquide spermatique de l'homme. On a trouvé des valeurs basses pour cette enzyme et une diminution significative au point de vue statistique a été observée dans les cas d'azoospermie.

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Condition	No. of cases	Spermatozoa number (millions/ml)	Ceruloplasmin ( $\mu\text{g}/100\text{ ml}$ ) <sup>a</sup>
Normospermic	5	56–150	$594 \pm 87$
Oligospermic	11	0.5–40	$504 \pm 199$
Azospermic	5	0	$242 \pm 55$

<sup>a</sup> Mean  $\pm$  standard deviation.