this difference diminishes with time, suggesting a precursor relationship.

2. Labelling of the RNA in all cell fractions, except the cytoplasmic fraction, is quickly inhibited by actinomycin D. The inhibition of the labelling of the membrane RNA occurs rather more slowly than that of the 'nuclear' fractions.

3. There is no apparent physical difference between the 'nuclear' and cytoplasmic ribosomes. The higher specific radioactivity of the 'nuclear' ribosomes is due to the presence of more labelled ribosomal and messenger RNA. It is therefore suggested that the 'nuclear' ribosomes contain the initial products of nuclear RNA synthesis.

4. At early times of labelling, only the 16s RNA of the 30s ribosome sub-unit is labelled, but at later times the 23s RNA of the 50s ribosome sub-unit becomes labelled.

5. Studies of the incorporation of ^{14}C -labelled amino acids into protein confirmed previous observations that the cytoplasmic membrane is an important site of protein synthesis in the cell.

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REFERENCES

- Butler, J. A. V., Crathorn, A. R. & Hunter, G. D. (1958). *Biochem. J.* **69**, 544.
Butler, J. A. V. & Godson, G. N. (1963). *Biochem. J.* **88**, 176.

- Butler, J. A. V. & Godson, G. N. (1964). *Nature, Lond.*, **201**, 876.
Ceriotti, G. (1955). *J. biol. Chem.* **214**, 59.
Gierer, A. (1963). *J. molec. Biol.* **6**, 148.
Godson, G. N. (1962). Ph.D. Thesis: University of London.
Godson, G. N. & Butler, J. A. V. (1962). *Nature, Lond.*, **193**, 655.
Godson, G. N. & Butler, J. A. V. (1963). *Biochem. J.* **88**, 259.
Godson, G. N., Hunter, G. D. & Butler, J. A. V. (1961). *Biochem. J.* **81**, 59.
Gros, F., Gilbert, W., Hiatt, H., Attardi, G., Spahr, P. F. & Watson, J. D. (1961b). *Cold Spr. Harb. Symp. quant. Biol.* **25**, 111.
Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Riseborough, R. W. & Watson, J. D. (1961a). *Nature, Lond.*, **190**, 581.
Hunter, G. D., Brookes, P., Crathorn, A. R. & Butler, J. A. V. (1959). *Biochem. J.* **73**, 369.
Hunter, G. D. & Godson, G. N. (1961). *Nature, Lond.*, **189**, 140.
Kidson, C., Kirby, K. S. & Ralph, R. K. (1963). *J. molec. Biol.* **7**, 312.
Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
Kurland, C. G. (1960). *J. molec. Biol.* **2**, 83.
Levinthal, C., Keynan, A. & Higa, A. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1631.
McCarthy, B. J., Britten, R. J. & Roberts, R. B. (1962). *Biophys. J.* **2**, 27.
McQuillen, K., Roberts, R. B. & Britten, R. J. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 1437.
Riseborough, R. W., Tissières, A. & Watson, J. D. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 430.
Schlessinger, D. (1963). *J. molec. Biol.* **7**, 569.
Suit, J. (1963). *Biochim. biophys. Acta*, **72**, 488.
Tissières, A., Watson, J. D., Schlessinger, D. & Hollingworth, B. R. (1959). *J. molec. Biol.* **1**, 221.
Warner, J. R., Knopf, P. M. & Rich, A. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 122.

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A Study of the Steroid Metabolism of an Interstitial-Cell Tumour of the Testis

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Although there are many reports on the clinical aspects of virilizing interstitial-cell tumours of the testis, little information is available on the biochemical aspects of this disorder. Thus Savard *et al.* (1960) carried out clinical, morphological and bio-

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chemical studies on a boy with an interstitial-cell tumour by using radioactive substrates. However, the blood concentrations of steroids were not reported in their extensive investigation. Dominguez (1961) reported a case of bilateral testicular tumour, of uncertain histological type, complicating congenital adrenocortical hyperplasia. Results

similar to those of Savard *et al.* (1960) were obtained by incubation experiments; in addition, 17 α -hydroxyprogesterone was detected in spermatic-vein blood.

A patient with a virilizing interstitial-cell tumour of the testis presented an opportunity to obtain further information on the relation between the steroid production of the tumour *in vitro* and the blood concentration of certain steroids. The present study was therefore undertaken in addition to clinical and morphological investigations (J. M. Bayer, H. Breuer, R. Buchholz, J. Cervós-Navarro, W. Nocke, G. Schriefers, E. R. Smith & E. Tonutti, unpublished work).

MATERIALS AND METHODS

Patient. A 3½-year-old boy with an interstitial-cell tumour of the right testis was operated on for removal of the tumour, during which time samples of blood were obtained from the spermatic vein associated with the tumour, and from a cubital vein.

Extraction of steroids from blood. Blood was centrifuged for 10 min. at 2000g, and to the plasma obtained ethanol (4 vol.) was added slowly with stirring; the protein precipitate was removed by centrifugation (10 min. at 2000g) and washed with ethanol (4 vol.). The combined ethanolic extracts were evaporated to dryness *in vacuo* at 40°, and the residue was taken up in water (25 ml.) and extracted with benzene (25 ml.) four times. The combined benzene extracts were evaporated to dryness *in vacuo* at 40° and the residue was partitioned between light petroleum (b.p. 40–70°) (25 ml.) and ethanol–water (7:3, v/v) (25 ml.). The ethanolic solution was evaporated to dryness *in vacuo* at 40°, and the residue dissolved in benzene (25 ml.); this solution was extracted twice with NaOH (4%, w/v) (25 ml.) and the extract used for estimation of oestrogens. The benzene solution was washed free of alkali with water and evaporated to dryness as before.

The dry residue of the benzene solution was dissolved in benzene (2 ml.) and transferred to a column (2 g.; 13 mm. diam.) of alumina (4.5% moisture content; Savory and Moore Ltd.), which was then washed with benzene (25 ml.). Two fractions were eluted with ethanol–benzene: EB-1 [with 25 ml. of ethanol–benzene (1:499, v/v)] and EB-2 [with 20 ml. of ethanol–benzene (1:49, v/v)]. These two eluates were evaporated to dryness before paper chromatography. The papers (Schleicher and Schüll, no. 2043b Mgl) were washed by running in an ethanol–benzene (1:9, v/v) system for 24 hr. immediately before use. The two residues, EB-1 and EB-2, were chromatographed in the Bush-type system *n*-heptane–benzene–methanol–water (10:5:12:3, by vol.) until the solvent front had reached 40 cm. Ultraviolet-absorbing substances were located by contact photography. These zones of paper were cut out and the substances eluted by allowing ethanol–benzene (1:9, v/v) (approx. 1 ml.) to seep through the paper and drip into an enclosed vessel. The material from the spermatic-vein blood that corresponded in *R_F* to testosterone was rechromatographed on paper impregnated with propylene glycol–methanol (1:1, v/v), after allowing the methanol to evaporate; the mobile phase was benzene–cyclohexane.

The paper was dried overnight at approx. 60°, and the ultraviolet-absorbing substances were again located by contact photography.

Tumour tissue. The brown-coloured tumour, weighing approx. 10 g., was taken immediately to the Laboratory and cooled to 4°. Approx. 6 g. of the tumour was used in the present study; it was sliced by the method of Deutsch (1936).

Incubation. In a typical experiment, the slices (200 mg.) were incubated in Krebs–Ringer phosphate–saline (Krebs & Henseleit, 1932), pH 7.4 (5 ml.), containing glucose (20 mM), for 60 min. at 37° in air with agitation. When steroids were added to the incubation mixture, androst-4-ene-3,17-dione (200 µg.) was dissolved in propylene glycol (0.1 ml.), and 21-hydroxypregn-4-ene-3,20-dione (200 µg.) was dissolved in Krebs–Ringer phosphate–saline (2 ml.). Where trophic hormones were added, human chorionic gonadotrophin (Primogonyl; Schering, West Berlin) (300 i.u.) or adrenocorticotrophic hormone (Acethropan; Hoechst, Frankfurt-am-Main, Germany) (5 i.u.) was dissolved directly in the incubation mixture.

Extraction of steroids from incubation mixture. The mixture was extracted three times with ether–chloroform (3:1, v/v) (1 vol.), and the solvents were removed under reduced pressure. The residue was dissolved in a small volume of ethanol for transfer to paper. The C₁₉ steroids were chromatographed on washed Schleicher and Schüll paper no. 2043 Mgl in the solvent system cyclohexane–benzene (1:1, v/v) on propylene glycol-impregnated paper at 20°, and finally dried at 50° for 24 hr. The C₂₁ steroids were chromatographed on washed Whatman no. 1 paper in the solvent system cyclohexane–toluene–methanol–water (10:10:17:3, by vol.) at 25°. Ultraviolet-absorbing substances were located on chromatograms by contact photography, and eluted with methanol.

Determination of 17-oxo steroids. 17-Oxo steroids were determined by the Zimmermann reaction as modified by Callow, Callow & Emmens (1938).

Determination of α -ketols. α -Ketols were determined by reaction with triphenyltetrazolium chloride (Schriefers & Korus, 1956). 17 α -Hydroxy corticosteroids were determined by the method of Porter & Silber (Flood *et al.* 1961).

Optical measurements. Ultraviolet absorption was measured with a Beckman model DU spectrophotometer, with 10 mm. quartz cells. The infrared-absorption spectra were measured with a Beckman IR 5 spectrophotometer, by using the KBr micro-technique (approx. 80 µg. of substance and 10 mg. of KBr). To purify the substance for infrared spectrophotometry, it was chromatographed on a column of washed Celite (6 g.; 3 ml. of stationary phase) with the solvent system cyclohexane–toluene–methanol–water (5:5:7:3, by vol.).

RESULTS

Identification of steroids in blood

Spermatic-vein blood. A total of 37.5 ml. of plasma was obtained from 65 ml. of blood drawn, during approx. 7.5 min., from the spermatic vein. The two extracts EB-1 and EB-2 from this plasma were chromatographed in the system *n*-heptane–benzene–methanol–water and seven ultraviolet-

absorbing spots (VS-1 to VS-7) were found. VS-1 (R_f 0), VS-3 (R_f 0.3), VS-5 (R_f 0.46) and VS-7 (R_f 0.85) were not identified. VS-2 (R_f 0.14) corresponded to 11β -hydroxyandrost-4-ene-3,17-dione, and after elution from the paper it showed ultraviolet absorption, $\lambda_{\max}$ 240 $m\mu$. The material was sublimed and gave crystals, m.p. 200°; the m.p. of the authentic compound was 199–200°. VS-4 (R_f 0.37) corresponded to testosterone and was twice rechromatographed on paper impregnated with propylene glycol, with cyclohexane–benzene as mobile phase. Two ultraviolet-absorbing spots were found, VS-4a with R_f 0.36 corresponding to testosterone, and VS-4b with R_f 0.42 corresponding to 17α -hydroxyprogesterone. The substances eluted from these two spots showed $\lambda_{\max}$ 240 $m\mu$. Further evidence for the identification of VS-4a as testosterone was provided by incubating it with the microsomal fraction of human placenta: oestradiol- 17β was obtained as a metabolite. VS-6 (R_f 0.71) corresponded to androst-4-ene-3,17-dione; after elution from the paper this substance showed ultraviolet absorption, $\lambda_{\max}$ 240 $m\mu$, and gave a positive Zimmermann reaction.

Peripheral-vein blood. A total of 33 ml. of plasma was obtained from 62 ml. of blood taken from a cubital vein at the same time as the spermatic-vein sample was drawn. The two extracts were chromatographed in the system *n*-heptane–benzene–methanol–water, and one ultraviolet-absorbing spot (R_f 0.85) found which was not identified. The zones of paper corresponding in R_f to 11β -hydroxyandrost-4-ene-3,17-dione (P-2), testosterone (P-4) and androst-4-ene-3,17-dione (P-6) were eluted; there was no detectable ultraviolet absorption at 240 $m\mu$ in any of these eluates.

Quantitative determination. The amounts of steroids found in the plasma samples are given in

Table 1. The results are calculated on the basis of the observed molecular extinction coefficients of the authentic substances. The linearity of the ultraviolet absorption of the peripheral-blood extracts indicates the absence of Δ^4 -3-oxo steroids. This also appears to justify the application of Allen's (1950) correction to the ultraviolet absorption of the extracts from spermatic-vein blood.

Incubation experiments with tumour tissue

Experiment with glucose. Tumour-tissue slices were incubated in the presence of human chorionic gonadotrophin in Krebs–Ringer phosphate–saline, containing glucose as the only substrate. The extracts obtained after incubation were chromatographed on paper impregnated with propylene glycol, with cyclohexane–benzene as mobile phase. Five ultraviolet-absorbing spots (G-1 to G-5) were found, two of which (G-1, R_f 0; and G-5, R_f 0.9) were not further investigated. G-2 had the same R_f as authentic 11β -hydroxyandrost-4-ene-3,17-dione; after elution from the paper it showed $\lambda_{\max}$ 240 $m\mu$ and gave a positive Zimmermann reaction ($\lambda_{\max}$ 510 $m\mu$). G-3 had the same R_f as testosterone and showed $\lambda_{\max}$ 240 $m\mu$ after elution from the paper. The R_f of G-4 was identical with that of androst-4-ene-3,17-dione; after elution, G-4 showed $\lambda_{\max}$ 240 $m\mu$ and gave a positive Zimmermann reaction ($\lambda_{\max}$ 510 $m\mu$).

Experiment with androst-4-ene-3,17-dione. The extracts obtained after the incubation of androst-4-ene-3,17-dione with tumour slices in the medium as used in the glucose experiment were chromatographed in the same system as above. Four ultraviolet-absorbing zones (A-1 to A-4) were detected which had the same R_f values as 11β -hydroxyandrost-4-ene-3,17-dione (A-1), testosterone (A-2), androst-4-ene-3,11,17-trione (A-3) and androst-4-

Table 1. *Ultraviolet absorption of and amounts of steroid in fractions obtained from plasma of spermatic-vein blood (VS) and cubital-vein blood (P)*

Extinctions were measured in ethanol solutions (2.5 ml., except VS-6 where 5 ml. was used); the light-path was 10 mm. $E_{\text{corr.}} = E_{240\text{ m}\mu} - \frac{1}{2}(E_{220\text{ m}\mu} + E_{260\text{ m}\mu})$.

Fraction	Steroid	$E_{220\text{ m}\mu}$	$E_{240\text{ m}\mu}$	$E_{260\text{ m}\mu}$	$E_{\text{corr.}}$	Steroid ($\mu\text{g.}$)	
						In sample	In 100 ml. of plasma
VS-2	11β -Hydroxyandrost-4-ene-3,17-dione	0.252	0.319	0.096	0.145	11.1	29.5
VS-4	Testosterone + 17α -hydroxyprogesterone	0.303	0.602	0.181	0.360	Not calculated	
VS-4a	Testosterone	0.091	0.117	0.042	0.051	3.7	10.0
VS-4b	17α -Hydroxyprogesterone	0.171	0.325	0.102	0.189	14.7	39.0
VS-6	Androst-4-ene-3,17-dione	0.241	0.491	0.122	0.309	39.5*	105.0
P-2	—	0.029	0.021	0.014	–0.001	—	—
P-4	—	0.067	0.044	0.030	–0.005	—	—
P-6	—	0.037	0.027	0.017	0.000	—	—

* A value of 40 $\mu\text{g.}$ was obtained by the Zimmermann reaction.

Table 2. *Steroids found after incubation of tumour-tissue slices, alone and in the presence of androst-4-ene-3,17-dione or 21-hydroxypregn-4-ene-3,20-dione*

Details are given in the text.

Steroid incubated	Addition	Steroids found	Amount of steroid recovered ($\mu\text{g.}/200 \text{ mg. of tissue} \times \text{hr.}$)
None	Human chorionic gonadotrophin (300 i.u./200 mg. of tissue)	11 β -Hydroxyandrostenedione	6.3
		Testosterone	—
		Androst-4-ene-3,17-dione	2.7
Androst-4-ene-3,17-dione	Human chorionic gonadotrophin (300 i.u./200 mg. of tissue)	11 β -Hydroxyandrost-4-ene-3,17-dione	15
		Testosterone	5
		Androst-4-ene-3,11,17-trione	5
		Androst-4-ene-3,17-dione	—
21-Hydroxypregn-4-ene-3,20-dione	Adrenocorticotrophic hormone (5 i.u./200 mg. of tissue)	11 β ,21-Dihydroxypregn-4-ene-3,20-dione	78
		17 α ,21-Dihydroxypregn-4-ene-3,20-dione	4
		21-Hydroxypregn-4-ene-3,20-dione	123
	None	11 β ,21-Dihydroxypregn-4-ene-3,20-dione	70
		17 α ,21-Dihydroxypregn-4-ene-3,20-dione	4
		21-Hydroxypregn-4-ene-3,20-dione	137

ene-3,17-dione (A-4) respectively. All four substances, after elution from the paper, showed absorption with λ_{max} at 240 μ . In addition, substances A-1, A-3 and A-4 gave a positive Zimmermann reaction (λ_{max} 510 μ).

Experiment with 21-hydroxypregn-4-ene-3,20-dione. The extracts obtained after the incubation of 21-hydroxypregn-4-ene-3,20-dione with tumour slices in Krebs-Ringer phosphate-saline, containing glucose, were subjected to paper chromatography in the system cyclohexane-toluene-methanol-water. Three ultraviolet-absorbing spots (D-1 to D-3) were detected which corresponded in mobility to 11 β ,21-dihydroxypregn-4-ene-3,20-dione (R_f 0.19), 17 α ,21-dihydroxypregn-4-ene-3,20-dione (R_f 0.29) and 21-hydroxypregn-4-ene-3,20-dione (R_f 0.73). For further characterization, D-1 was chromatographed on a Celite column, with the same solvent system as above. From the appropriate fractions of the eluate, a crystalline product was obtained which showed the same infrared-absorption spectrum as authentic 11 β ,21-dihydroxypregn-4-ene-3,20-dione. This experiment was repeated in the presence of adrenocorticotrophic hormone (5 i.u./200 mg. of tissue), and the same metabolites were found.

Quantitative determination. The amounts of steroids produced during the incubation of tumour tissue with the various substrates are summarized in Table 2.

DISCUSSION

The testis tumour used in the present study showed the typical histological signs of a Leydig-cell adenoma (see Plate 1). The tumour did not contain adrenocortical cells, as judged by the absence of spongiocytes, which are regularly found in normal and pathological tissue of adrenal-cortical origin (J. M. Bayer, H. Breuer, R. Buchholz, J. Cervós-Navarro, W. Nocke, H. Schriefers, E. R. Smith & E. Tonutti, unpublished work). In view of this finding, it is of considerable interest that the tumour was able to carry out 11 β -hydroxylation of androst-4-ene-3,17-dione and 21-hydroxypregn-4-ene-3,20-dione *in vitro*. So far, 11 β -hydroxylation has been regarded as characteristic of adrenocortical tissue. Additional evidence for the 11 β -hydroxylase activity in the tumour was provided by the presence of 11 β -hydroxyandrost-4-ene-3,17-dione in spermatoc-vein blood.

The tumour tissue also contained 17 α -hydroxylase activity as shown by the conversion of 21-hydroxypregn-4-ene-3,20-dione into 17 α ,21-dihydroxypregn-4-ene-3,20-dione *in vitro* and by the presence of 17 α -hydroxyprogesterone in the spermatoc-vein blood. Further, the activity of two hydroxy steroid dehydrogenases was detected by the conversion of androst-4-ene-3,17-dione into testosterone (17 β -hydroxy steroid dehydrogenase)

Plate 1. Section of testis tumour (magnification $\times 315$), stained with haematoxylin-phosphomolybdic acid-orange G-aniline blue. The tumour tissue consists of large uniform sharply-defined cells held together in rounded groups by strong connective tissue. The nuclei are nearly spherical with large nucleoli, and frequently are not centrally placed; they vary in size but no striking irregularities occur. Almost all nuclei are surrounded by clouds of weakly-staining very fine granules, but the periphery of the cells is clear. At the edge of the granular cloud, in most cells, are coarse darkly-staining inclusions strung together to form a boundary. Yellow-brown pigment occurs rarely, in single cells, in contrast with the striking dark-brown macroscopic appearance of the tissue. Reinecke crystals and lipoid droplets are absent. The tumour tissue lay entirely within the tunica albuginea, and contains no tubules. Structures typical of the adrenal cortex are absent, as are spongiocytes and sinusoids, which occur frequently in adenomata of the adrenal cortex. Considerable expansion of the interstitial connective tissue was noticeable.

and by the conversion of 11β -hydroxyandrost-4-ene-3,17-dione into the corresponding 11-oxo compound (11β -hydroxy steroid dehydrogenase).

None of the steroids found in the spermatic-vein blood could be detected in the peripheral blood under similar experimental conditions. However, metabolites of the steroids found were excreted in the urine in elevated amounts (J. M. Bayer, H. Breuer, R. Buchholz, J. Cervós-Navarro, W. Nocke, H. Schriefers, E. R. Smith & E. Tonutti, unpublished work). Thus the secretion of 11β -hydroxyandrost-4-ene-3,17-dione by the tumour can be correlated with a five- to ten-fold increase in the urinary excretion of the total 11-oxygenated 17-oxo steroids. A similar increase in the urinary excretion of androsterone and aetiocholanolone was associated with the androst-4-ene-3,17-dione secreted by the tumour. Pregnane-3 α ,17 α ,20 α -triol, a metabolite of 17α -hydroxyprogesterone, was found in urine in amounts that exceeded post-operative values by about 50-fold.

The experiment with glucose alone permits the calculation of the production rate *in vitro* of the tumour as a whole. Thus the whole tumour would be expected to have produced 40 μ g. of 11β -hydroxyandrost-4-ene-3,17-dione and 17 μ g. of androst-4-ene-3,17-dione in 7.5 min. The spermatic-vein blood collected in 7.5 min. contained 11 μ g. of 11β -hydroxyandrost-4-ene-3,17-dione and 40 μ g. of androst-4-ene-3,17-dione. The production rate *in vitro* and the production rate *in vivo*, both expressed as the sum of the two steroids, are in good agreement. However, the 11β -hydroxyandrost-4-ene-3,17-dione:androst-4-ene-3,17-dione ratio is greater in the experiment *in vitro* than in the experiment *in vivo*; possibly this is due to the protracted action of the 11β -hydroxylase on the androst-4-ene-3,17-dione formed during incubation.

No oestrogens could be detected in either of the blood samples, and also no oestrogens were detected in the experiment *in vitro* with androst-4-ene-3,17-dione as substrate. Thus no aromatizing enzyme activity could be demonstrated in the tumour.

Insofar as they can be compared, the present results confirm the findings of Savard *et al.* (1960). These authors used radioactive substrates and found that the tumour tissue *in vitro* was capable of synthesizing steroids from acetate, of hydroxylating steroids in the 11β , 17α - and 21-positions as well as of carrying out oxidoreductions at positions 11β and 17β . The present results cannot be com-

pared with those of Dominguez (1961) since the tumours studied were of different types.

It still remains obscure whether the 11β -hydroxylating activity is a property of the interstitial cells that differs quantitatively from the characteristic activity associated with adrenocortical tissue, and becomes manifest only when a neoplasm is formed.

SUMMARY

1. The production of certain steroids *in vivo* and *in vitro* has been studied in an interstitial-cell tumour of the testes.

2. Tumour tissue possessed the following enzyme activities: 11β -hydroxylase, 17α -hydroxylase, 17β -hydroxy steroid dehydrogenase and 11β -hydroxy steroid dehydrogenase.

3. Androst-4-ene-3,17-dione, 11β -hydroxyandrost-4-ene-3,17-dione testosterone and 17α -hydroxyprogesterone were found in spermatic-vein blood from the tumour, but not in peripheral blood.

4. The rate of formation *in vitro* of androst-4-ene-3,17-dione plus 11β -hydroxyandrost-4-ene-3,17-dione agreed with the rate of secretion by the tumour into the spermatic vein.

5. The results are briefly discussed in relation to the output of steroids in the urine of the patient.

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REFERENCES

- Allen, W. M. (1950). *J. clin. Endocrin.* **10**, 71.
- Callow, N. H., Callow, R. K. & Emmens, C. W. (1938). *Biochem. J.* **32**, 1312.
- Deutsch, W. (1936). *J. Physiol.* **87**, 53P.
- Dominguez, O. V. (1961). *J. clin. Endocrin.* **21**, 663.
- Flood, C., Layne, D. S., Ramcharan, S., Rossipal, E., Tait, J. F. & Tait, S. A. (1961). *Acta endocr., Kbh.*, **36**, 237.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Savard, K., Dorfman, R. I., Baggett, B., Fielding, L. L., Engel, L. L., McPherson, H. T., Lister, L. M., Johnson, D. S., Hamblen, E. C. & Engel, F. L. (1960). *J. clin. Invest.* **39**, 534.
- Schriefers, H. & Korus, W. (1956). *Naturwissenschaften*, **43**, 517.