Regulation of Urinary Steroid Excretion

1. EFFECTS OF DEHYDRO*ISO*ANDROSTERONE AND OF ANTERIOR PITUITARY EXTRACT ON THE PATTERN OF DAILY EXCRETION IN MAN

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(*Received* 20 *December* 1948)

In the course of investigations concerned with the steroid metabolism of mental patients, attempts were made for therapeutic purposes to influence the pattern of daily excretion of 17-ketosteroids and of cortin. The effects thereby produced throw some light on the regulatory mechanisms governing steroid metabolism. The question of how these processes are related to the mental state will be dealt with in later papers. This communication is concerned with the biochemical investigations alone, and deals with the actions of dehydro*iso*androsterone and an anterior pituitary lobe extract.

EXPERIMENTAL

All the patients investigated were chronic schizophrenics in which the clinical picture was of similar form and duration. Estimations of the urinary 3(α)- and 3(β)-hydroxy-17-ketosteroids, total oestrogen and cortin were carried out before treatment, and were later repeated at least once after treatment had commenced. One group of patients was given intramuscular dehydro*iso*androsterone (50 mg./injection dissolved in 2·5 ml. ethyl oleate), and the other group was given Ambinon, a pituitary anterior lobe extract containing about 50 i.u. gonadotrophic hormone and 100–200 Heye-Laqueur guinea pig units thyrotrophic hormone/ml. (1 ml./injection).

All urines were stored at 0° without preservative, and worked up as soon as possible after completion of collection. Creatinine estimations were performed on every separate 24 hr. specimen as a test for completeness; any specimens showing gross departures from the appropriate norm of daily excretion were rejected.

17-Ketosteroid and oestrogen estimations. These were carried out on pooled 48 hr. specimens. A suitable portion (2·0–2·5 l.) of the urine was treated in the cold with 20 ml. conc. HCl/l., then brought to the boil and boiled vigorously for 10 min. After cooling, it was transferred to a large continuous liquid-liquid extractor. Extraction with benzene was carried out for a minimal period of 12 hr., and the extract was then removed and worked up according to the procedure of Callow, Callow, Emmens & Stroud (1939).

After the benzene solution containing the neutral fraction had been separated, washed and dried over Na_2SO_4, one fifth of the volume was taken and evaporated to dryness on an oil bath at 100° (the use of a water bath was avoided to prevent contamination by water), the last traces of benzene being removed by a vacuum pump. After redissolving in 5 ml. ethanol, the total 17-ketosteroids were estimated by the Zimmermann reaction, under the conditions described by Callow, Callow & Emmens (1938). Of the remaining ketosteroid solution in benzene, a portion containing 5 mg. was removed, evaporated to dryness, and separated into ketonic and non-ketonic fractions by a micro modification of the separation procedure employed by Callow & Callow (1938), using the Girard reagent T (13 mg. Girard reagent T in 0·1 ml. glacial acetic acid were used for 5 mg. ketosteroid dissolved in 0·1 ml. acetic acid). The separated ketonic fraction was further separated into α and β fractions by digitonin precipitation as described by Frame (1944). 17-Ketosteroid estimations were made on the whole ketonic fraction and on the separate α and β fractions.

The phenolic fraction of the original benzene extract was, as usual, acidified, extracted with benzene, the extract dried, evaporated and finally brought into solution in arachis oil. The total oestrogen content was assayed by the rat method, using oestradiol as standard preparation, and is expressed in arbitrary 'rat units', one rat unit being equivalent to approx. 0·023 μg. oestradiol. The total activity of the extracts was insufficient to permit accurate statistical evaluation.

Cortin estimations were carried out by an application of the method described by Heard, Sobel & Venning (1946), but the following modifications were introduced: (*a*) $CHCl_3$ was used as the extracting solvent, and (*b*) the urine was extracted continuously in the cold in an apparatus similar to that employed by Robinson & Warren (1948). The urine sample, after passing through the apparatus and collecting in a suitable vessel, was returned to the funnel and passed through the solvent again. This process was repeated until twenty such extractions had been made. By this means, the disadvantage of emulsion formation was completely avoided. Trial extractions with aqueous solutions of deoxycorticosterone acetate confirmed that complete extraction could be effected in this way. (*c*) For the final estimation of the dried extract, the Hagedorn-Jensen estimation, as used by Hemphill & Reiss (1947) for blood cortin, was applied. For every batch of urines taken, a water blank was carried through simultaneously, to check the purity of the various reagents.

Reagents. Benzene was purified from technical material. It was distilled once, then washed three times with water, dried over Na_2SO_4, allowed to stand several days (at least 24 hr.) over alumina with occasional shaking, and finally distilled through a 6-pear column.

Ethanol was dried by treating with Ca turnings, and then further purified by heating for 1 hr. with 10 g./l. semicarbazide acetate, and recovering. Acetic acid was purified by the method of Orton & Bradfield (1927).

Table 1. *Daily excretion of steroids in urine of patients before and after hormone treatment*

Patient and clinical diagnosis	Date	17-ketosteroids: Total (mg./24 hr.)	Non-ketonic fraction (mg./24 hr.)	Ketonic fraction (mg./24 hr.)	α fraction (mg./24 hr.)	β fraction (mg./24 hr.)	α in ketonic (%)	β in ketonic (%)	Cortin (mg./24 hr.)	Oestrone (rat units/ 24 hr.)
		A. Patients treated with dehydro*iso*androsterone								
1. N.E.H., chronic schizophrenia	29. v. 48	4·7	2·0	2·7	2·25	0·45	83·5	16·5	1·19	<3
	29. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	5. vi. 48	14·7	2·1	12·6	9·5	3·1	75·3	24·7	0·84	<3
2. M.G.H., chronic schizophrenia	29. v. 48	7·6	2·3	5·3	4·4	0·90	83·3	16·7	1·19	<3
	29. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	5. vi. 48	25·2	4·0	21·2	14·8	6·4	69·7	30·3	0·99	—
3. P.F., chronic schizophrenia	29. v. 48	6·4	1·78	4·62	4·02	0·6	86·5	13·5	0·94	<3
	29. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	5. vi. 48	34·5	9·1	25·4	24·3	1·1	95·5	4·5	1·8	—
4. L.F., chronic schizophrenia	28. iv. 48	8·6	2·0	6·6	5·1	1·5	77·5	22·5	1·4	<4
	29. v. 48	8·3	2·5	5·8	4·5	1·3	77·2	22·8	1·38	<3
	29. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	5. vi. 48	39·7	1·7	38·0	30·4	7·6	79·9	20·1	1·26	—
5. W.C.T., chronic catatonic schizophrenia	24. v. 48	7·1	1·7	5·4	3·6	1·8	65·8	34·2	1·42	3
	27. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	1. vi. 48	13·6	2·1	11·5	7·33	4·17	63·7	36·3	2·60	—
6. G.R.B., catatonia	25. v. 48	5·0	1·55	3·45	2·53	0·92	73·3	26·7	1·5	3
	27. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	1. vi. 48	20·8	3·0	17·8	13·7	4·04	77·3	22·7	1·61	—
7. D.T., catatonia	25. v. 48	5·4	3·04	2·36	2·18	0·18	92·3	7·7	0·96	4
	27. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	1. vi. 48	15·3	3·7	11·6	7·9	3·7	67·8	32·2	0·86	3
	9. vii. 48	18·7	3·5	15·0	12·1	2·9	81·0	19·0	2·31	12
	15. vii. 48	21·8	3·0	18·8	15·5	3·3	82·4	17·6	1·07	4
	11. viii. 48	Injections stopped								
	24. ix. 48	6·8	1·6	5·2	4·56	0·64	87·6	12·4	—	—
8. R.C.D., chronic schizophrenia	25. v. 48	10·4	3·25	7·15	5·55	1·60	77·4	22·6	0·82	4
	27. v. 48	Commenced daily injections of 50 mg. dehydro*iso*androsterone								
	1. vi. 48	27·3	5·1	22·2	14·7	7·5	66·2	33·8	1·93	12
	6. vii. 48	32·8	1·6	31·2	20·6	10·6	66·0	34·0	3·6	8
	15. vii. 48	39·3	8·1	31·2	20·7	10·5	66·2	33·8	2·1	4
	11. viii. 48	Injections stopped								
	24. viii. 48	9·3	3·1	6·2	5·9	0·30	95·8	4·2	1·54	—
		B. Patients treated with Ambinon								
9. J.E.D., simple schizophrenia	26. iv. 48	11·4	2·0	9·4	8·08	1·32	86·0	14·0	1·53	3
	1. vii. 48	6·9	0·7	6·2	5·37	0·83	86·6	13·4	1·60	—
	6. vii. 48	Commenced daily injections of Ambinon								
	10. vii. 48	11·7	2·0	9·7	9·22	0·48	95·1	4·9	1·32	—
	17. vii. 48	5·9	1·6	4·3	3·98	0·32	92·5	7·5	—	24
	22. vii. 48	Injections stopped								
	26. viii. 48	7·5	2·3	5·2	4·8	0·40	92·3	7·7	—	<3
10. E.K., paranoid schizophrenia	23. vi. 48	13·7	3·4	10·3	7·3	3·0	71·0	29·0	1·63	—
	29. vi. 48	13·5	2·8	10·7	8·2	2·5	76·8	23·2	1·59	8
	6. vii. 48	Commenced daily injections of Ambinon								
	10. vii. 48	19·1	4·2	14·9	11·8	3·1	79·0	21·0	1·46	24
	17. vii. 48	17·8	2·6	15·2	12·1	3·1	79·4	20·6	—	24
	16. viii. 48	12·9	3·1	9·8	6·5	3·3	66·0	34·0	—	—
	22. viii. 48	Injections stopped								
	27. viii. 48	15·2	4·3	10·9	8·4	2·5	76·5	23·5	—	6
11. J.A.L., chronic schizophrenia	5. v. 48	5·1	0	5·1	3·6	1·5	69·9	30·1	0·94	4
	12. v. 48	6·3	2·6	3·7	3·39	0·31	91·7	8·3	0·83	—
	12. vi. 48	7·6	3·2	4·4	3·91	0·49	88·8	11·2	1·03	6
	14. vi. 48	8·9	4·0	4·9	4·74	0·16	96·7	3·3	0·93	—
	27. vi. 48	Commenced daily injections of Ambinon								
	6. vii. 48	4·1	1·3	2·8	2·64	0·16	94·2	5·8	1·15	4
	15. vii. 48	2·4	—	—	—	—	—	—	0·44	4
12. A.J.A., chronic schizophrenia	29. vi. 48	16·2	4·3	11·9	11·03	0·87	92·7	7·3	2·00	no response
	6. vii. 48	Commenced daily injections of Ambinon								
	10. vii. 48	9·0	2·9	6·1	5·7	0·40	93·7	6·3	1·54	>6
	17. vii. 48	5·1	1·3	3·8	3·55	0·25	93·4	6·6	—	—

RESULTS

The results for the whole series of estimations are recorded in Table 1. It will be seen that the administration of the 3(β)-hydroxy-17-ketosteroid dehydro-*iso*androsterone caused a considerable rise in the excretion of the α fraction of 17-ketosteroids in all of the eight patients receiving this preparation. In every case, except one, the β fraction was also increased, but not to the same extent as the α fraction. Cortin was increased in three cases (nos. 3, 7 and 8) and total oestrogen was increased in two cases (nos. 7 and 8).

Ambinon caused a reduction in the α fraction of 17-ketosteroids, accompanied in most cases by a definite rise in total oestrogen. No marked changes in cortin excretion were seen in these cases.

DISCUSSION

The remarkable increase in the excretion of α fraction of 17-ketosteroids following administration of dehydro*iso*androsterone may be compared with similiar phenomena reported by other workers. Dorfman, Wise & Shipley (1948) demonstrated increased 3(α)-hydroxy-17-ketosteroid excretion in one patient after the injection of a 3(β)-hydroxy-17-ketosteroid (*iso*androsterone). Mason & Kepler (1947) also studied the ketosteroid excretion of two patients suffering from Addison's disease who received over 1000 mg. of dehydro*iso*androsterone during 12 days, administered while the adrenal insufficiency was controlled by treatment with deoxycorticosterone and sodium chloride. Subsequent examination of the urine revealed no dehydro*iso*androsterone, but only androsterone, aetiocholanolone, and other 3(α)-hydroxy-ketosteroids, which accounted for 43 and 19 % respectively of the injected hormone. These authors assumed that dehydro*iso*androsterone is the precursor of the α-steroids mentioned, under normal conditions. It is, however, debatable whether this increase of α-steroid excretion is due to a direct chemical conversion of the β-steroid, as Dorfman *et al.* (1948) and Mason & Kepler (1947) assume. One has to account for the fact that the 50 mg. dehydro-*iso*androsterone injected daily resulted in increases of total ketonic fraction in the range of 12–64 % of the injected dose only in the first days after commencing the dosage. Moreover, the simultaneous increase of cortin excretion in three cases and of total oestrogen in two cases are hardly to be accounted for by a purely chemical mechanism. Little is known as yet about the physiological action of dehydro*iso*-androsterone on the gonads or adrenals, but it is feasible that a stimulation of the adrenal cortex or gonadal elements by this substance may have taken place; a more detailed examination of this possibility may well prove to be of considerable clinical importance.

The remarkable increase in cortin excretin in one case, and the slight but significant rise in two cases might point to some stimulatory influence on the production of C_{21} steroids. It is known that injection of deoxycorticosterone brings about a reduction in adrenal cortical function, with decreased hormone production. Since dehydro*iso*androsterone is produced for the most part in the adrenal cortex, it is possible that in some patients the endogenous production is reduced when the compound is administered, thereby increasing the availability of the natural precursor for the production of C_{21} steroids. It is possible that competition between production of dehydro*iso*androsterone and of cortin may exist; a regular inverse relationship between cortin excretion and β-steroid excretion has in fact been observed in these laboratories in a patient showing cyclic changes of activity and depression (full details of this work will be published later). During depression the β-steroid excretion was increased, and the cortin excretion decreased, and the reverse tendency was shown during normal and maniacal phases. In this connexion it is of some interest to note that the patient who showed the greatest rise in cortin excretion after dehydro*iso*androsterone was the only one who showed complete mental improvement, with subsequent discharge from hospital.

Regarding the decreased α-steroid excretion (which was preceeded in two cases by an increase) brought about by Ambinon, it may be assumed that this is due to the gonadotrophic fraction contained in this preparation. Previous work (Carreyett, Golla & Reiss, 1945) has shown that gonadotrophic hormone prepared from pregnant mare serum decreases the total ketosteroid output in patients, and depletes the lipid content of rat adrenals, after several days' treatment. It is thus evident that dehydro*iso*-androsterone and Ambinon produce opposite effects on the excretion of 3(α)-hydroxy-17-ketosteroids. It remains to be seen, however, to what extent they are antagonistic in the strict sense.

SUMMARY

1. Administration of dehydro*iso*androsterone produced in several mental patients a marked rise in the excretion of the 3(α)-hydroxy-17-ketosteroids. Increases of cortin and of oestrogens were noted in a few cases.

2. The anterior pituitary-lobe extract, Ambinon, produced a net fall in the excretion of the 3(α)-hydroxy-17-ketosteroids, usually accompanied by a rise in total oestrogen excretion.

3. Possible mechanisms for the changes described above are discussed.

The authors wish to acknowledge their indebtness to Organon Laboratories Ltd., for supplies of dehydro*iso*androsterone and Ambinon preparations, to Messrs W. G. Warren, D. J. Dell and Miss B. Binham for technical assistance, and to Mr F. Knight for valuable co-operation in the collection of urine specimens.

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The Use of the Waring Blender in Biochemical Work

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(*Received* 2 *July* 1948)

In recent years the Waring blender has found widespread application in biochemical work, owing to the ease and speed with which it disintegrates all sorts of heterogeneous materials into uniform suspensions. It is surprising that the literature contains no reference, so far as the authors are aware, to possible harmful effects of treatment in the Waring blender on the properties of biological material. Several workers seem to have noticed that the intense aeration occurring in operating the blender causes oxidation of reducing substances. This can be gathered from the fact that they use the blender *in vacuo* or in an atmosphere of inert gas. However, no definite information on this point seems to have been published. A further possibility of damage to biological material might be anticipated, viz. denaturation of proteins and hence inactivation on enzyme systems through intensive agitation.

While carrying out experiments on the reducing matter and oxidizing enzymes of wheat germ and other mill streams, the authors found that even short treatment in the Waring blender greatly affected these systems. The present paper describes these findings which were first reported at the New Zealand Science Congress held in Wellington in 1947. In the meantime Quinlan-Watson & Dewey (1948) reported inactivation of cytochrome *c* oxidase caused by treating animal tissue in the Waring blender. The main work on oxidizing enzymes of wheat will be the subject of separate papers.

EXPERIMENTAL

Quantitative evidence for the effect of the Waring blender on suspensions of various mill streams comes from the following experiments.

Effects of the Waring blender on reducing matter

Suspensions of wheat germ (e.g. 1 part germ and 11 parts of water) were made (*a*) by grinding with sand and water in a mortar, (*b*) by treating in the Waring blender, and (*c*) by boiling for 2 min. In the blender, the temperature of a 250 g. suspension rose by 18° within 5 min. owing to the generation of heat by friction. To avoid injurious increases of temperature on treating the suspensions for more than 5 min. they were cooled after 5 min. stirring, or ice water was used for making them.

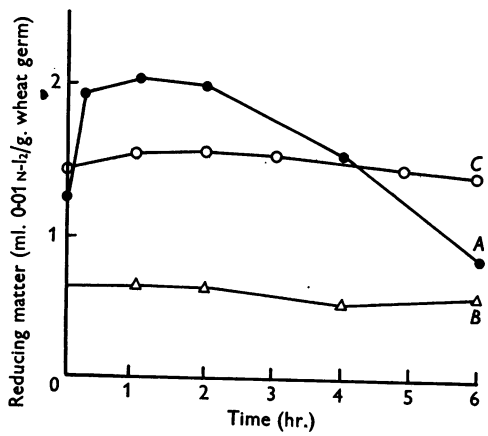

Fig. 1. Changes of reducing matter in wheat-germ suspensions obtained *A*, by grinding with sand; *B*, by 5 min. treatment in the Waring blender; *C*, by boiling and allowing to stand at 40° for several hours.

The suspensions were then kept in a water bath at 40°, and samples for determination of reducing matter were withdrawn at various intervals. The results were expressed as ml. 0·01 N-I_2 solution/g. of germ. The changes of reducing matter with time are shown in Fig. 1. These graphs in the