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Regulation of Urinary Steroid Excretion

2. SPONTANEOUS CHANGES IN THE PATTERN OF DAILY EXCRETION IN MENTAL PATIENTS

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The daily excretion of 17-ketosteroids of a large series of mental patients has been estimated for several months—in some cases for more than a year. It was observed that in some cases the rate of daily excretion was fairly constant, but in others there were wide variations, far outside the possible error of determination. Some light has been thrown on the physiological mechanism responsible by a detailed long-term study of steroid excretion in one patient with cyclical manic and depressive phases, and by the study of diurnal variations in 17-ketosteroid output in chronic schizophrenic patients before and after treatment.

EXPERIMENTAL

Estimation of steroid excretion. In all cases where the total neutral 17-ketosteroid fraction only was required, the simultaneous hydrolysis and extraction procedure of Callow, Callow, Emmens & Stroud (1939) was employed. These estimations were carried out at intervals over long periods, on 24–72 hr. urine specimens of mental patients and normal subjects. In the case of the cyclic patient, however, the more detailed procedure for fractionation and estimation of the steroids, as previously described by us (Reiss, Hemphill, Gordon & Cook, 1949), was followed.

Diurnal variations were estimated by making use of the micromethod of Drekter, Pearson, Bartczak & McGavack

(1947) which requires only 10 ml. urine; such estimations were carried out in duplicate, the margin of error being rather greater than in the macromethod. It was thus possible to estimate 17-ketosteroid output on urine collections taken every 3 hr. throughout the day (7 a.m.–10 p.m.), ending with a 9 hr. night collection (10 p.m.–7 a.m.).

RESULTS

Variations in 24 hr. excretion. Fig. 1 illustrates the excretion rates of total 17-ketosteroids in mg./24 hr. for normal subjects, over periods of 6–14 months, and this diagram may be compared with the corresponding excretion rates for chronic schizophrenic patients, which are recorded in Fig. 2. The excretion rates for normal subjects are relatively constant, varying at the most ± 1.5 mg./24 hr. Some of the patients also show excretion rates which approach this constant pattern (e.g. nos. 7, 9, 10 and 12), but others show wide variations, which may amount to ± 6.0 mg./24 hr. These variations could not necessarily be correlated with the clinical picture. Further investigations designed to ascertain whether relationships exist between psychotic condition and constancy or inconstancy of ketosteroid excretion are at present being undertaken.

Fig. 1. Variation of urinary steroid excretion in normal subjects (72 hr. urine specimens).

Fig. 2. Variation of urinary steroid excretion in schizophrenic subjects (48 hr. urine specimens).

Fig. 3. Variations of urinary steroid excretion in a case of manic-depressive psychosis. *A*, total 17-ketosteroids (mg./24 hr.); *B*, ketonic fraction of 17-ketosteroids (mg./24 hr.); *C*, 3(β)-hydroxy-17-ketosteroids (% of ketonic fraction); *D*, oestrogens (rat units/24 hr.); *E*, cortin, mg./24 hr.; *Dp*, times of change into depression; *Mn*, times of change into mania.

Fig. 3 records the daily excretion rates of various steroid fractions for a 45-year-old typical manic-depressive psychotic patient, with relatively short-phase cycles. Manic phases varied between 1 and 5 weeks, and depressive phases between 6 and 24 days, the transition from one state to another being

always rapid. The remission or interval of normality between manic and depressive phases was never more than a few days and merged gradually into the succeeding phase. Values are recorded for excretion of total 17-ketosteroids, ketonic fraction, percentage 3(β)-hydroxy-17-ketosteroids in the ketonic

fraction, cortin and oestrone, estimated at intervals over a period of 1 year (dates of transition from phase to phase are included). The inconstancy of the excretion rates of all these fractions is particularly marked in this case, the possible magnitude of the variations being greater than in the other cases, for corresponding periods. In as short a period as 10 days, changes of 100–300 % could occur; the cortin excretion, for instance, changed during each cycle by over 100 %, as also did that of 3(β)-hydroxy-17-ketosteroids. During the course of the year, a total 17-ketosteroid excretion varying between 2 and 18 mg./24 hr. was observed. A closer examination of the variations in different fractions shows an inverse relationship between changes in β -steroid and cortin

a tendency to maximal values during the hours shortly after waking, followed by a regular decline during the rest of the day. This normal pattern was shown by only a few of the psychotic patients. The results are illustrated in Fig. 4. The following deviations from the normal may be noted: (1) A greatly enhanced increase in the 'waking' output (7–10 a.m.) compared with the night output, sometimes amounting to 400 %, the corresponding figure for normal subjects being 60 % (Pincus & Hoagland, 1943). (2) Slight variations only during the day and night. (3) Maximal excretion delayed until a later time during the day, instead of in the 7–10 a.m. period. (4) A second peak in output occurring late in the day, in addition to the morning peak.

Fig. 4. Diurnal variations of urinary 17-ketosteroid excretion.

excretion—a similar inverse relationship between β -steroid excretion and oestrone excretion was also sometimes noted. The effect was most marked in the first regular cycles, where the onset of depression appeared to be accompanied by a tendency to excretion of a high proportion of β -steroids and a low cortin excretion. Cortin excretion was usually rising at the transition into mania, when at the same time the proportion of β -steroids was falling. The clear-cut pattern of inverse relationship was followed by an irregular psychological period, during which time changes in ketosteroid excretion continued, unaccompanied by psychological changes. From September onwards, a long period of depression was maintained, in which the average β -steroid excretion was continuously high (over 20 %) and the cortin excretion comparatively low.

Diurnal variations. The variations in 17-ketosteroid excretion during the day and night were investigated in normal individuals by Pincus (1943), who reported minimal excretion during sleep and

Fig. 5. Influence of dehydroisoandrosterone on diurnal pattern of 17-ketosteroid excretion.

Types 1 and 2 are both influenced by dehydroisoandrosterone treatment, as illustrated in Fig. 5. Patient 2, a catatonic of 20 years' standing, underwent no change in mental state after the treatment, and it will be seen that his diurnal variation curve, which is of type 2, was not materially different after treatment had stopped, though it underwent considerable change during the course of the treatment. Patient 1, on the other hand (a hallucinated and restless schizophrenic of 16 years' standing), whose diurnal pattern is of type 1, responded well to treatment, and was subsequently discharged from hospital; at the same time, the diurnal pattern was definitely changed after cessation of treatment, approximating more to the normal.

DISCUSSION

A scrutiny of the ketosteroid excretion values of the psychotic patients, considered in relation to the known facts concerning their clinical condition,

showed that those whose excretion was constant over several months (nos. 5, 7, 8, 9, 10 and 12) were older chronic schizophrenic patients (two of them catatonic) who had been in hospital for at least 10 years. The diurnal curves of this group, as far as they have been investigated, are of type 2, showing little variation throughout the 24 hr. period. The other patients referred to in Fig. 2 were restless and hallucinated. This group showed much sharper changes in the diurnal pattern, with a degree of variation several times greater than the normal maximum of 60% observed by Pincus & Hoagland (1943) and by us. In some, the excretion peak was delayed, while in others, more than one peak could be observed. It remains to be seen whether the differentiation of diurnal curves into the types 1, 2, 3 and 4 may become useful for diagnostic purposes. It is clear, however, that the resultant of the sharply changing diurnal curves illustrated by types 1, 3 and 4 is likely to produce the greater variations in daily output.

The diurnal variation of 17-ketosteroid excretion is an expression of the adaptation by the individual to the demands of his environment and daily life. Very little adrenal cortical activity is necessary during sleep, and the most essential demand in the 24 hr. cycle is in the process of waking. It is, therefore, understandable that the excretion is at a maximum for normal subjects during the first few hours of the morning, and this adaptation mechanism is evidently disturbed in schizophrenic patients.

The daily excretion pattern of the manic-depressive patient shows that different functions of the adrenal cortex need not change in a parallel manner, since the cortin-like substances can show increases at the same time as the 3 (β)-hydroxy-17-ketosteroids are decreased, and vice versa. The antagonism between these two components was regularly observed, and changes in their values nearly always took place at times corresponding with changes in the mental state. The sudden change into depression took place while the β -steroid excretion was rising, or at a maximum, at the same time as the cortin excretion was falling; the opposite changes were seen at the commencement of the maniacal phase. For one period during the summer months of the year the typical excretion pattern was unaccompanied by the usual mental changes. Towards the autumn of the year the patient went into a prolonged depression, during which, for more than 3 months, the β -steroids remained high and the cortin excretion comparatively low.

It is important to consider the extent to which such changes in adrenal cortical activity are a primary cause, or a secondary consequence of the mental disturbance. In this connexion, the work of Allen, Broster, Vines, Patterson, Greenwood, Marrian & Butler (1939) may be taken into account.

These workers claimed that paranoid psychotics with adrenogenital virilism, who showed increased ketosteroid excretion, were improved mentally after unilateral adrenalectomy, when the ketosteroid level was also lowered. These are undoubtedly special cases, and while we do not suggest that the psychiatric condition was similar to those we have studied, the physiological mechanism for the production of some of the symptoms is probably the same. Mental changes after castration are generally known, and considerable disturbances in testicular function in schizophrenic patients were demonstrated by Hemphill, Reiss & Taylor (1944). Further, in the previous paper of this series (Reiss *et al.* 1949) there was some indication that the 3 (β)-hydroxy-17-ketosteroid, dehydroisoandrosterone, could influence the mental state. In the present study, the diurnal curve became more similar to the normal after administering dehydroisoandrosterone in the case of the patient whose mental condition improved, but not in the case of the patient who did not improve. It may be added that, in recent months, we have accumulated results of investigations, to be described later, on depressed patients showing abnormally high β -steroid excretion, some of whom improved mentally after treatment with testosterone (which also brought about a decreased β -steroid excretion). Hoagland (1944) reported improvement in work efficiency after administration of pregn-5-en-3-ol-20-one. It is also pertinent to remember that steroid hormones may influence the brain circulation (Reiss & Golla, 1940) and may also exert a narcotic-like action (Selye, 1941).

The facts quoted above suggest that changes in hormone production may directly influence the mental state. It may be argued, however, that the response of the adrenal cortex to different demands is itself directed by vegetative brain centres, via the pituitary anterior lobe. It is perhaps permissible to visualize a cycle of events in which a disturbance of certain vegetative brain centres results in a dysfunction of the adrenal cortex—the latter in its turn influencing other brain parts and producing mental change. Further clarification of such a mechanism must await the accumulation of considerably more clinical and experimental evidence.

SUMMARY

1. Daily 17-ketosteroid excretion in the urine of mental patients, estimated at intervals for several months, showed either constant or widely varying values.

2. Different types of diurnal variation of 17-ketosteroid excretion have been recorded in mental patients.

3. A patient suffering from manic-depressive psychosis was found to excrete a high proportion of 3 (β)-hydroxy-17-ketosteroids, accompanied by a

relatively low cortin value, during most of the depressive phases. The reverse changes were usually observed during manic phases.

4. The possible relationships between steroid hormone production and mental symptoms are discussed in the light of the above findings.

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Optical Rotation of the Molybdate Complex of *Dextro-iso*Citric Acid

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Owing to the high optical rotatory power of the *isocitrate-citrate-molybdate* complex, milligram quantities of *dextro-isocitric acid* can be determined polarimetrically (Martius, 1938; Jacobsohn, 1940; Martius & Leonhardt, 1943; Krebs & Eggleston, 1943).^{*} The accuracy of the method depends, *inter alia*, on information concerning the specific rotation of this complex under the conditions of the test. Previous measurements, giving an $[\alpha]_D$ value of -780° , could not be regarded as highly accurate as the *isocitric acid* used for the measurements was not pure (Krebs & Eggleston, 1943). Dr H. B. Vickery has recently given us a sample of pure dimethyl *isocitrate lactone* prepared from the *isocitric acid* of *Bryophyllum* leaves (Pucher, Abrahams & Vickery, 1948). This enabled us to make more precise measurements of the rotation of the complex in the conditions used for the quantitative determination of *isocitric acid*.

EXPERIMENTAL

To prepare a stock solution of *dextro-isocitric acid* 202 mg. (1 mmol.) of the dimethyl ester lactone were dissolved in 20 ml. 0.25 N-NaOH and kept at 40° for 30 min. This treat-

ment was found to be sufficient for the quantitative conversion of the substance into free *isocitric acid*. The stock solution was diluted as required.

The standard test conditions were as follows (see Krebs & Eggleston, 1943). To 10 ml. of the solution to be tested were added 1.25 ml. glacial acetic acid, 2.5 ml. M-sodium citrate and 11.25 ml. 29% (w/v) ammonium molybdate. (Citrate was included because it increases the rotation of the complex (Krebs & Eggleston, 1943; Martius & Leonhardt, 1943).) After mixing and filtration the solution was transferred to a 2 dm. water-jacketed Hilger polarimeter tube. Rotation readings were taken at different temperatures. The light source was a sodium lamp.

The results of the measurements at different temperatures are given in Table 1. The values for $[\alpha]_D$ are calculated for anhydrous *isocitric acid*, not for the molybdate complex (the structure of which is unknown). The concentration of *isocitric acid* in the test solution, as given in the top line of Table 1, refers to the concentration of the solution in the polarimeter tube, i.e. after the addition of the various reagents.

The data show that $[\alpha]_D$ decreases with rising temperature and also, slightly, with rising concentration of *isocitric acid*. It is therefore important in polarimetric determinations to control the temperature and to select an $[\alpha]_D$ figure for the calculation which is valid for the concentration range under test.

Further data for $[\alpha]_D^{20^\circ}$ at ten different concentrations of *isocitric acid* are shown in Table 2. It should be noted that the absolute error of the measurements is constant (about $\pm 0.02^\circ$ for α), and that the percentage error in α is therefore greater the smaller the value of the reading.

^{*} The term '*dextro-isocitric acid*' is used in accordance with the conventions laid down in *Principles of Abstracting* (Bureau of Abstracts, 1948). The substance has previously been called '*d-isocitric acid*' (Pucher, Abrahams & Vickery, 1948) and '*(-)-isocitric acid*' (Krebs & Eggleston, 1944).