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).

Table 1. *Rotation of the dextro-isocitrate-citrate-molybdate complex*

(For test conditions see text; α_D is the rotation in degrees observed in a 2 dm. tube, $[\alpha]_D$ the specific rotation calculated for isocitric acid.)

Concentration of isocitric acid (mg./ml.) ...	Temp. (°)	0.48		0.96		1.92		3.84	
...		α_D	$[\alpha]_D$	α_D	$[\alpha]_D$	α_D	$[\alpha]_D$	α_D	$[\alpha]_D$
	10	-0.80°	-834°	-1.56°	-812°	-2.98°	-776°	-5.39°	-701°
	15	-0.77°	-802°	-1.50°	-781°	-2.82°	-735°	-5.18°	-675°
	20	-0.75°	-781°	-1.43°	-745°	-2.66°	-693°	-4.96°	-645°
	25	-0.73°	-760°	-1.38°	-719°	-2.51°	-654°	-4.75°	-618°
	30	-0.70°	-729°	-1.32°	-688°	-2.35°	-612°	-4.54°	-591°
	35	-0.68°	-709°	-1.26°	-657°	-2.19°	-570°	-4.33°	-564°

Table 2. *Effect of the concentration of dextro-isocitric acid on $[\alpha]_D$ in solutions containing the isocitrate-citrate-molybdate complex*

(Standard conditions. 2 dm. tubes. 20°. $[\alpha]_D$ calculated for isocitric acid.)

isocitric acid concentration (mg./ml. solution tested)	α_D observed	$[\alpha]_D$
0.240	-0.40°	-834°
0.480	-0.75°	-781°
0.576	-0.89°	-772°
0.672	-1.02°	-758°
0.768	-1.15°	-750°
0.864	-1.29°	-747°
0.960	-1.43°	-745°
1.44	-2.09°	-726°
1.92	-2.66°	-693°
3.84	-4.96°	-645°

In the previous experiments (Krebs & Eggleston, 1943) the rotation measured was about -1.80° (2 dm. tubes) at

approximately 18° . According to Table 1 $[\alpha]_D^{18^\circ}$ for these conditions is -735° , whilst it was previously assumed to be -780° . A small correction of the previously calculated isocitric acid values is therefore required. The equilibrium mixture of citrate, isocitrate and *cis*-aconitate had been taken to contain 6.2% isocitrate (at 38°). The recalculation gives a value of 6.6% isocitrate.

SUMMARY

The optical rotatory powers of solutions containing the isocitrate-citrate-molybdate complex have been measured under varying conditions, in order to obtain reference values for the polarimetric determination of dextro-isocitric acid.

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The Use of Buffered Columns in the Chromatographic Separation of 2:4-Dinitrophenyl Amino-acids

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During an investigation of the action of papain on wool keratin, the elegant method of Sanger (Sanger, 1945; Porter & Sanger, 1948) was used to determine the end-groups of the peptides liberated by the enzyme. In this method, the terminal amino groups of proteins or polypeptides are condensed with 1:2:4-fluorodinitrobenzene and the amino-acids originally carrying free amino groups are isolated

chromatographically as their coloured *N*-2:4-dinitrophenyl (DNP) derivatives after acid hydrolysis.

It was observed at an early stage that silica gels which had been successfully employed for the separation of *N*-acetyl amino-acids were of little use for the separation of DNP amino-acids. Using chloroform as the solvent, for example, practically