in their formulation of the oxidation of the dihydro-derivative by potassium ferricyanide. But whereas the change studied by them appears to take place in two stages, the formula of the lactoflavin derivative readily accommodates a single-stage process, for it is well known that $\alpha\beta$ -unsaturated ketones, $R_2C = \overset{\cdot}{C} - \overset{\cdot}{C} = O$, exhibit kationoid reactivity, for example, towards cyanide ions, at the β -carbon atom. Applying this to the $-N = \overset{\cdot}{C} - \overset{\cdot}{C} = O$, or, perhaps better, to the $-N = \overset{\cdot}{C} - \overset{\cdot}{C} = N - \overset{\cdot}{C} = O$, grouping in lactoflavin, we have

The change is completed by addition to the 1-nitrogen atom of the proton from the original hexose hydrate.

These considerations also point to a possible interpretation of the function of the methyl groups in the lactoflavin molecule, which is so far not understood. For the grouping in question might also be expected to exhibit the familiar property of nitro-groups in promoting anionoid attack in the ortho- and para-positions of the benzene nucleus. On the other hand, methyl groups in the nucleus, and obviously especially so in the para-position, militate against such an attack, and thus tend to localize it on the nitrogen atom. Accordingly, whereas 9-*d*-1'-ribitylisoalloxazine itself exhibits no vitamin B₂ effect⁴, its 7-methyl derivative does⁵. The influence of other alkyl groups or of methyl groups in other positions has not yet been recorded.

J. KENNER.

College of Technology,
Manchester.

¹ *Ber.*, B, 65, 705 (1932).

² For example, Warburg and Christian, *Helv. chim. Acta*, 19, E81, (1936).

³ *Helv. chim. Acta*, 19, 811 (1936).

⁴ Kuhn and Weygand, *Ber.*, B, 66, 166 (1933).

⁵ Karrer and others, *Helv. chim. Acta*, 18, 908 (1935).

Corticosteron, a Crystallized Compound with the Biological Activity of the Adrenal-cortical Hormone

In 1929-30 it was found that the cortex of the adrenal gland produces a hormone essential for life¹. Several attempts have since been made to isolate it in pure form.

Kendall *et al.*² announced (1934) the isolation of crystals (m.p. 210°) which were claimed to have the activity of the hormone. They later wrote³ that the problem was more complex, that their earlier described crystals were a mixture, and that for complete hormonal activity at least two components were essential. In their latest communications⁴ they report the isolation of several compounds (*A-E*) one of which (compound *E*, m.p. 201°-208°) "showed definite cortical hormone activity when tested on the rat (Ingle's method)" but that "the quantitative activity of compound *E* is much less than of cortin itself".

Wintersteiner and Pfiffner⁵ described six different

crystallized compounds (*A-F*), all of which were inactive when tested on adrenalectomized dogs by the method of Swingle and Pfiffner⁶.

Reichstein⁷ reported the isolation of nine substances (*A-I*), eight of which were found inactive in doses of 0.5-2 mgm. in rats (method of Everse-de Fremery⁸). Only the ninth, substance *H*, has in the meantime been found to be active. This substance, however, was not quite pure. After the elimination of a small amount of a new, high melting substance (substance *M*, m.p. 205°-208°), a pure compound in beautiful crystals was obtained from the remainder. It shows to a large extent the biological activity of the raw material and is called corticosteron (m.p. 180°-182° (corr.), $[\alpha]_D^{25} = +223^\circ$, $c = 1.12$ in abs. alcohol). With the exception of a few details the chemical

constitution has been elucidated; the formula will be reported in the near future.

Adrenalectomized rats require a daily dose of 0.5-1 mgm. corticosteron per rat (method of Everse-de Fremery). This amount represents the approximate rat unit and is the biological equivalent of 1 c.c. of commercial 'cortin' (corresponding to 50 gm. of fresh gland) for rats. The experiments on bilaterally adrenalectomized dogs gave similar equivalent values, but since the method of testing used was somewhat different than that of Swingle and Pfiffner, the activity cannot be expressed in terms of dog units.

The curative and preventive properties were tested on four dogs (body weight ranging from 11 kgm. to 18 kgm.) which had been adrenalectomized $\frac{3}{4}$ -1 $\frac{1}{2}$ years previously. During this period, they received a daily oral dose of 0.3-0.4 gm. sodium chloride per kgm. body weight and the necessary amount of commercial 'cortin' (2.5-5 c.c. per dog per day). Without the salt, about ten times as much 'cortin' was necessary. The complete withdrawal of 'cortin' caused the animals to fall into insufficiency, even if the salt dose were considerably increased. In the curative tests, three to six times the preventive doses of 'cortin' were necessary to bring the animals back to normality from their state of insufficiency. In the various experiments on dogs, an average range of 0.25-0.5 mgm. corticosteron was found to be equivalent to 1 c.c. of the standard 'cortin' solution.

These experiments show that corticosteron is a highly active cortical hormone, but the existence of other active or at least activating components is still possible.

Amsterdam and Oss,
and
Zurich.
Nov. 28.

P. DE FREMERY.
E. LAQUEUR.
T. REICHSTEIN.
R. W. SPANHOFF.
I. E. UYLDERT.

¹ Rogoff, Stewart, *J. Amer. Med. Assoc.*, 92, 1569 (1929). Swingle, Pfiffner, *Science*, 71, 321 (1930); *Medicine*, 11, 731 (1932); Hartman, Brownell, *Science*, 72, 76 (1930).

² *Proc. Staff Meet. Mayo Clinic*, 2, 245 (1934).

³ *J. Amer. Med. Assoc.*, 105, 1486 (1935); see also ref. 4.

⁴ *Proc. Staff Meet. Mayo Clinic*, 11, 351 (1936); *J. Biol. Chem.*, 114, 613 (1936).

⁵ *J. Biol. Chem.*, 111, 599 (1935); 114, lxxx (1936).

⁶ Pfiffner, Swingle, Vars, *J. Biol. Chem.*, 104, 701 (1934).

⁷ *Helv. chim. Acta*, 19, 29, 1107 (1936).

⁸ *Acta Brev. Neerl.*, 2, 152 (1932).