Considering the biological functions and structure of cozymase as well as dihydro-cozymase, it might be suggested that dihydro-cozymase is capable of being split enzymatically[5] at position (1) to adenine nucleotide and nicotine nucleotide and that the enzyme nucleotidase dephosphorylates the mono-nucleotides. Alternatively it might also be suggested that dihydro-cozymase is split at position (2) forming adenosin-diphosphoric acid, which is dephosphorylated by adenylpyrophosphatase present in the enzyme preparation. In either of these cases it seems probable that dephosphorylation of dihydro-cozymase, on account of its dibasic character, is facilitated more than dephosphorylation of monobasic cozymase.

No definite statement can be made as to the implications of the present observation until further investigations have been carried out.

Nucleotidase was prepared from intestinal mucosa according to Klein[6] and was purified by isoelectric precipitation at *p*H 4·7. The precipitate was dissolved in alkaline water and dialysed overnight.

Cozymase free from adenylic acid was reduced with sodium hydrosulphite at *p*H 7·2–7·4, and the excess of hydrosulphite was removed by aeration. The resulting dihydro-cozymase was carefully maintained at *p*H 7·6–8·0. The amount of dihydro-cozymase was estimated spectrophotometrically.

The experiments were carried out *in vacuo*, the substrate being added after evacuation in order to prevent re-oxidation of dihydro-cozymase and the reaction was stopped by 4 per cent CCl_3COOH without letting air enter the reaction mixture before it was acidified. Veronal buffer at *p*H 7·6 has always been used in the reaction mixture. It has been found that the dephosphorylation of dihydro-cozymase was not due to sulphate present. The parallel experiments with cozymase and dihydro-cozymase were done with the same cozymase preparation under identical conditions. Under the same experimental conditions, dephosphorylation of adenylic acid is about four times as large as that of oxidized cozymase.

The following table shows typical results :

Reaction time. (min.)	Purity of cozymase per cent	γ P/mgm. cozymase	γ P/mgm. dihydro-cozymase
30	100	18·75	34·0
30	100	31·25	50·0
15	90	16·12	35·64
15	90	17·12	26·14
30	90	25·75	44·68

N. B. DAS.
H. v. EULER.

Biochemical Institute,
University of Stockholm.
Feb. 26.

[1] Euler and Myrbäck, *Hoppe-Seylers Z.*, 177 (1928).
[2] Euler and Günther, *Hoppe-Seylers Z.*, **243**, 1 (1936).
[3] Euler and Heiwinkel, *Naturwiss.*, **25**, 269 (1937).
[4] Euler, Adler, Günther and Hellström, *Hoppe-Seylers Z.*, **245**, 217 (1937).
[5] Euler and Adler, *Hoppe-Seylers Z.*, **252**, 41 (1938).
[6] Klein, W., *Hoppe-Seylers Z.*, **207**, 125 (1932).

Standardization of Gonad-stimulating Hormones

IN view of a recent publication by Hamburger and Pedersen-Bjergaard[1] relating to methods of standardization of the gonad-stimulating hormones, the following observations made in these laboratories may be of interest.

I am in complete agreement with Hamburger and Pedersen-Bjergaard's contention that the corpus luteum method in mice and the vaginal smear method in rats are the most accurate available for standardization of the urinary gonad-stimulating hormone (Gonan, Physex, etc.). Formerly, it seemed to me more logical to use the corpus luteum method[2], since I was engaged on problems in which this phase of activity of the hormone was of particular interest. With the widening of the scope for such preparations to include even treatment of male gonadal dysfunction this argument disappears. I now standardize Gonan in terms of its ability to produce œstrus in 50 per cent of immature rats (40–50 gm. in groups of not less than ten), when given over a period of $2\frac{1}{2}$ days. Smearing is begun $1\frac{1}{2}$–2 days after the last injection, if the vagina is open. The ratio of this rat unit to the mouse unit (luteinizing) has been found to be about 1 : 12 ; this compares with a 1 : 15 ratio found by the Danish workers.

With regard to standardization of the hormone of pregnant mares' serum (Serogan, Antex), I am not in such complete agreement with these workers. In the first place, it is interesting to note that they consider the rat a more suitable animal than the mouse when the criterion of activity is increase of ovarian weight. They give the average weight of a pair of infantile mouse ovaries as 2·2 mgm. ; in these laboratories I find a figure of 1·7 mgm. for our stock and, although I work with minimum groups of ten, I feel that dissection cannot be carried out with sufficient accuracy with organs of this size, and I invariably prefer to work with rats when adopting this criterion of activity.

It is, however, generally recognized that the gonad-stimulating hormone of the serum of pregnant mares is predominantly follicle-stimulating (or œstrus-producing), and it seems to me that the vaginal smear technique is therefore a logical one to use, apart from its (in my opinion) greater accuracy. With a view to the use of this method, I have therefore carried out a series of tests to determine the ratio of the mouse unit, that is, the amount required to double the weight of the ovaries in 4 days, to the rat unit, the definition of which is the same as described above in dealing with the gonad-stimulating hormone of human pregnancy urine. Groups of ten rats were used, and 1 rat unit was found to be about 1/10 mgm., the ratio R.U. : M.U. (as described above) being 1 : 2.

Finally, it will be observed that throughout this communication I have avoided using the term 'gonadotropic', which has been in common use for a considerable time. A recent letter from Dr. Parkes[3] has stimulated interest in the question of nomenclature, but I do not feel entirely satisfied with the suggestions made by him. I therefore prefer to adopt a system used for some years particularly by the American workers in this field, that is, to refer to the 'gonad-stimulating' hormones, until some general international agreement can be reached.

P. G. MARSHALL.

Biochemical Laboratories,
British Drug Houses, Ltd.,
Graham Street, N.1.
Feb. 18.

[1] Hamburger and Pedersen-Bjergaard, *Quart. J. Pharm.*, **10**, 662 (1937).
[2] Marshall, *Biochem. J.*, **27**, 626 (1933).
[3] Parkes, NATURE, **141**, 36 (1938).