

Table 2 shows the specific activity values of the phosphorylated amino acids and phosphopeptides. The values for the two main fractions of the cell wall are of the same order as the data obtained from the purified phosphorylated amino acid of the *L. casei* at the end of the lag phase.

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## Fluorescence of Steroids

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It has previously been shown that the Zimmermann reaction cannot be ascribed to the 17-ketosteroids. Thus, the only reaction available at present for the characterization of steroids is their irreversible transformation into lumisteroids<sup>1</sup>, *e. g.*, the more stable stereoisomers with the 18-methylgroup in *cis*-position to the hydrogen atom at carbon 14. This reaction, naturally occurring to all steroids, is induced by concentrated sulphuric or phosphoric acid (sometimes alkalies), and by UV light of wave lengths shorter than 3130 Å.

This fluorescence is generally not observed with other steroids than estrogens and D-substances. It depends on the complicated nature of fluorescence, being a combination of at least five phenomena

1. The absorption of light energy by the electrons of one atomic group.

2. The re-emission of light by this or another atomic group.

3. The transformation of energy from the absorbing to the re-emitting group by molecular collisions or intramolecularly.

4. Quenching of energy by other atomic groups transforming the quenched energy into heat or light of unsuitable frequencies or dissociation.

5. The re-absorption of the re-emitted light by the solutions.

Thus, the formula of Perrin<sup>2</sup> for the total intensity of fluorescence:

$$f = k_1 c \exp(k_2 c) = k_1 c \exp(c/c_{\max})$$

will only apply when absorbing and re-emitting groups are combined in the same molecule, and quenching or re-absorption may be neglected.

If the principal part of the light energy is absorbed by a molecule which is totally free from the re-emitting atomic group, the intensity of fluorescence is directly proportional to the concentration of this group *e. g.* an amount of fluorescence suitable for quantitative analysis in all regions of concentration. However, the intensity of the absorbed energy must be sufficient for re-emission as well as for quenching to take place. The fluorescence must be measured in a wave length region where the steroid solutions have no absorption. An absolute requirement is that the fluorescence is not induced by light of so high frequencies that it will cause dissociation or polymerisation of steroids, *e. g.* UV light.

Both the absorption and the fluorescence of a steroid solution will always be spread over a broad wave length region. The maximum of the fluorescence intensity will be recorded by the photo cell when the filter between the light source and the container for the fluorescent medium has transmitted all visible light absorbable by this medium. The filter in front of the photo cell should ideally transmit light of all wave lengths absorbed by the former filter. The only way to approach this ideal filter system is to use certain solutions. Those of nickel sulphate and potassium bichromate have been used in a special type of fluorimeter.

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