To sum up, the most important microbiological conversions of steroids, are listed in Table XVIII.

Zusammenfassung

Die methodischen Grundlagen der Umwandlung von Steroiden durch Mikroorganismen werden diskutiert, insbesondere die apparativen Erfordernisse, die Kulturbedingungen sowie der Nachweis und die Isolierung der Reaktionsprodukte. Die Umsetzungen mit Enzymen aus Mikroorganismen werden denjenigen mit Nebennieren-Enzymen gegenübergestellt.

Von mikrobiologischen Reaktionen stehen Hydrierungen, Dehydrierungen und besonders Hydroxylierungen an verschiedenen Stellen der Steroidmolekel im Vordergrund. Im weiteren wurde auch Abbau der Seitenkette von Pregnanderivaten, der mit Ringspaltung und/oder mit Dehydrierung in 1-Stellung einhergehen kann, festgestellt. Die Einführung dieser Doppelbindung wird speziell besprochen.

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Further Measurements on the Bioluminescence of the Seedlings

The introduction and the development of the photomultipliers in the technique of light detection has permitted the counting of individual photons corresponding to extremely feeble luminous fluxes[1].

This method of detection can be applied with advantage to the study of problems connected with feeble luminous emissions; photons come directly from molecules that take part in some reaction, chemical or biochemical for instance, and can be very useful in furnishing a clue to molecular processes.

By means of a very sensitive apparatus, some of us have detected recently the emission of light in the visible spectrum by various germinating plants[2]. The present work is concerned with specifying such preliminary results, discussing some further properties of luminescence, giving a quantitative comparison of the intensity of the emitted light for different plants and at various ages during the germination, and showing that the production of light is strictly connected with the vital functions of seedlings.

(1) The apparatus used in the present research work is the same described in previous papers[3]. It is possible with this dispositive to detect the light coming from a big emitting area or volume. The plants used for present experiments belong to graminaceous and leguminous families. The seedlings were grown in complete darkness in order to avoid the formation of chlorophyll which, by its luminescence, would have disturbed the measurement.

The seedlings grew in humid surroundings at a constant temperature of 25°C. Measurement were conducted both on whole plants and on cold water extract of the plants or of the separate organs.

The extract is made by grinding a constant quantity of seedlings (generally a few grams) or of their organs with a corresponding quantity of a phosphate buffer solution of pH 7·3 and centrifugating the ground mixture. This pH value corresponds to the optimum value of the luminescent intensity. The measurements are made on a constant volume (10 cm^3) of the transparent liquid obtained which is collocated very close to the photocathode of the phototube.

The absence of chlorophyll is checked by the lack of red fluorescence of chlorophyll in the extracts.

(2) A first measurement was made for the purpose of detecting the intensity of the light emitted by seedlings in well established physiological conditions.

For such purpose a few tenths of 8 days old seedlings were used, which were placed ordinately horizontally under the phototube in such a way as to cover a surface of about 100 cm^2.

Light intensity emitted by seedlings in physiological conditions

	Pulses/min
Phototube backgrounds . .	8 000
Wheat	41 000
Beans	38 000
Lentils	22 000
Corn	15 000
Beans cut into pieces . . .	84 000

In the Table, the results obtained are set down. Both the plant and the phototube were at room temperature (20°C) throughout the measurement.

The results show clearly the existence of bioluminescence, and the activity observed is much greater than the thermoelectronic background of the photomultiplier. We should remark that the background is very stable and reproducible for a period of months.

The measurements repeated many times show a good reproducibility in a factor 2, but a measurement of this kind does not make possible a precise comparison

[1] R. W. ENGSTRÖM, J.O.S.A. *37*, 420 (1947). – G. A. MORTON and J. A. MITCHEL RCA Rev. *9*, 632 (1948). – R. WESTOO and T. WIEDLING, Ark Fysik *1*, 269 (1949).

[2] L. COLLI and U. FACCHINI, Nuovo Cimento *12*, 150 (1954).

[3] L. COLLI and U. FACCHINI, Nuovo Cimento *12*, 150 (1954). – L. COLLI, U. FACCHINI, and A. ROSSI, Nuovo Cimento *11*, 255 (1954).

of the intensities of light produced by various seedlings by reason of the absorption of light inside the seedling itself.

The last line of the Table shows that the activity of the cut seedlings multiplies two- or threefold,in comparison with that for of a normal one.

Later we carried out measurements to compare the luminescence intensity of various seedlings of different ages, by using the cold water extract previously described.

It was found that the light intensity of the extracts is reduced in the course of time to about one third beginning from the fifteenth to the thirtieth minute after starting of the operations of preparation; thereafter it remains sufficiently constant for hours.

Both the manner of intensity diminution as well as the further behaviour were found to depend on the quantity of air contained in the liquid.

By repeating the preparatory operation in a regular manner, a good reproducibility of the results was obtained, and therefore the average number of pulses per minute between the fifteenth and thirtieth minute was considered as a convenient index of the intensity of light emitted by the sample.

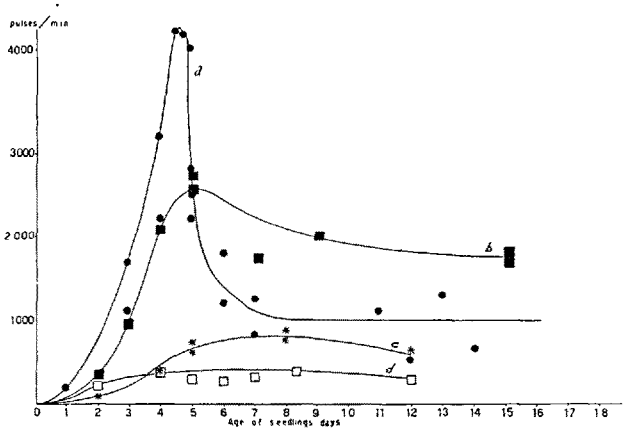

Fig. 1.—Luminescence intensity of seedlings versus the age of the seedlings. The ordinate represents the average light intensity between the 15th and the 30th min after the starting of the preparation obtained in standard conditions. The values of ordinates must be multiplied by 16.—*a* Beans; *b* Lentils; *c* Wheat; *d* Corn.

On Figure 1 are given the average intensities for various plants at various ages until the fifteenth day of their growth. The measurements were made on samples of equal weight and therefore the numbers of pulses of Figure 1 indicate the light emission of weight units of the seedlings (specific emission intensity).

As will be noted, the specific emission increases rapidly after the second day and reaches a maximum more or less around the fifth day.

The two leguminous plants studied have a higher specific activity than the two graminaceous plants.

In Figure 2 the specific emission of extracts of various organs of lentil seedlings at different ages are shown. It will be seen that the roots have a much greater specific light intensity than the other organs.

In Figure 3 the dependence of the luminescence on the pH value of the solution is shown. The values are obtained with seeds of lentils.

Finally, the spectral distribution of the emitted light was studied in respect to various plants, both as extracts as well as entire seedlings, by means of a series of coloured Wratten filters. Figure 4 shows the results, the values having been corrected for the photocathode yield.

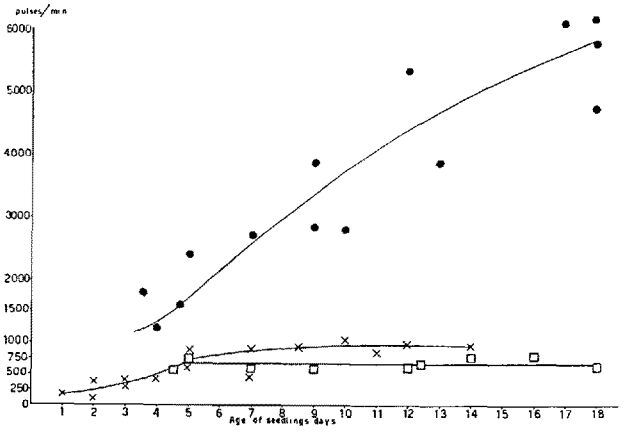

Fig. 2.—Luminescence intensity of extracts of various organs of lentils versus the age of the seedlings. Ordinates as in Figure 1.—× seeds; □ stems; ● roots.

(3) The sum of these results indicates with certainty the connection of the light emission with the vital functions of the plant. The interpretation of the empirical behaviour reported above is not possible at this early stage of the research; we think that these results will be of aid in understanding the nature of the luminescent reaction.

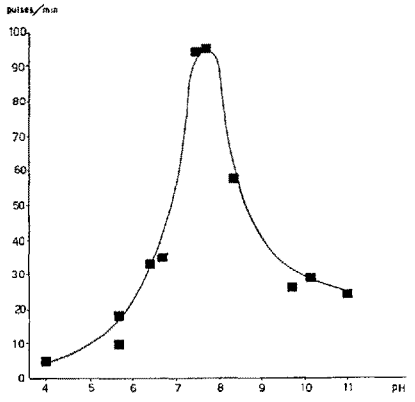

Fig. 3.—Luminescence intensity of extracts of lentil seads versus pH values.

It should be noticed that we examined some other seedlings, finding in any one case more or less intense light emission; it seems that the luminescent reaction is common to many kinds of seedlings.

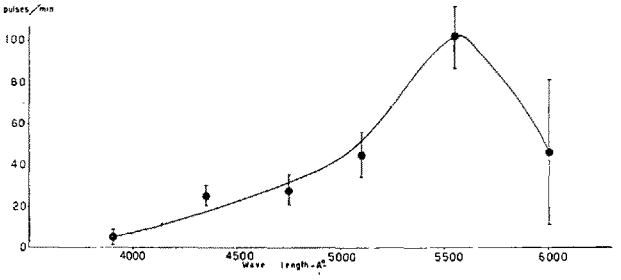

Fig. 4.—Luminescence spectrum: the points represent in arbitrary scale the average values obtained in various conditions, and with various kinds of seedlings.

The subject of our next work will be the results of biochemical investigations conducted on luminescent extracts for the purpose of ascertaining the nature of the enzymatic reaction which is responsible for the luminescence.

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Laboratori CISE, Milano, July 4, 1955.

Riassunto

Alcuni di noi recentemente hanno messo in evidenza un'emissione di luce di debole intensità nello spettro visibile, da parte di semi in germinazione. E argomento del presente lavoro discutere alcune accurate misure di intensità luminosa condotte su semi germinanti e su loro estratti acquosi in varie condizioni di età della plantule da 1 a 20 giorni. Viene anche studiata la dipendenza dell'emissione luminosa dal pH della soluzione e viene indicata la distribuzione spettrale della luce emessa.

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Area and Degree of Occupation of the Surface of Precipitates of Barium Sulphate

Precipitates of $BaSO_4$ were prepared by pouring 2 l of a 0·1 *N* hot solution of H_2SO_4 into 2 l of a 0·1 *N* hot solution of $BaCl_2$, under constant stirring; stirring was continued for 1 h; then the precipitate of $BaSO_4$ was separated from the solution, washed with hot and cold twice distilled water, washed with ethanol, and stocked under ethanol.

Photographs of the precipitate were taken with the optical microscope (1200 ×) and the electron microscope (3000 × and 10,000 ×); the surface average particle diameter was 2·7 μ, corresponding to a specific surface area of 0·51 m^2/g. This value agrees with the value given by KOLTHOFF[1] (about 1 m^2/g), but not with that given by DE BROUCKÈRE[2] (about 80 m^2/g).

We then studied the exchange of Ba*-ions (i.e. radioactive Ba-ions) between a saturated solution of Ba^*SO_4 and precipitate of $BaSO_4$. The isotope used was Ba* 131, $\tau_{1/2}$ = 12 days, emitter of γ-rays, no β's. When AMERSHAM delivered the radioactive material, they warned us that the activity was 600 times higher than expected; this must be attributed to an error in the assumed neutron cross-section of Ba 130, a point which now is being investigated in AMERSHAM; for us it was a lucky circumstance, since it enabled us to solve our problem easily.

We shook 100 cm^3 of saturated solution of Ba^*SO_4 at 16°C, hence containing 0·000215 g of $BaSO_4$ per 100 cm^3, or $5{\cdot}54 \times 10^{17}$ Ba-ions, with 100 mg precipitate of $BaSO_4$, and measured the γ-radio activity of 4 cm^3 of the solution after 0, 0·5, 1, 2, 3, 5, 10, 20, and 35 min. The results are given in Figure 1; a correction for background has been applied; we attribute the slow decrease at higher time periods to an exchange with the inward part of the lattice of $BaSO_4$; we therefore extrapolate this part of the curve back to $t = 0$ in order to obtain the true equilibrium between surface and precipitate; in this way we find: For the original solution at $t = 0$: 2829 counts/min. For the solution when equilibrium is reached, correction applied for exchange with the inward part of the lattice : 1919 counts/min.

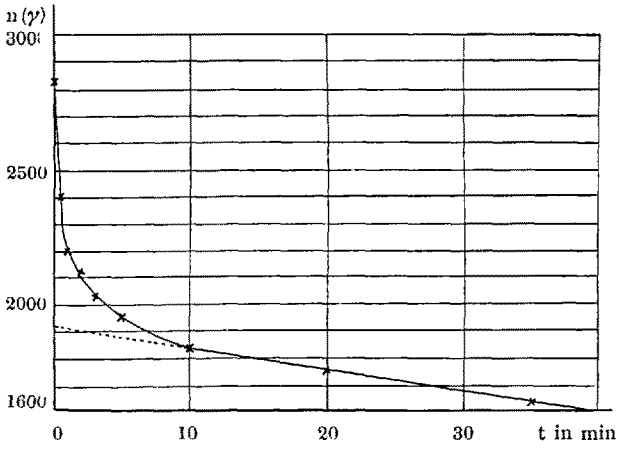
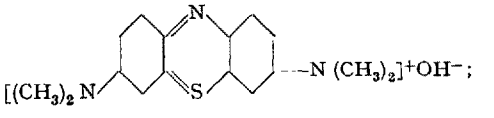


Fig. 1. – Number of γ-counts/min of 4 cm^3 of the solution as a function of the time of shaking.

From this we calculate that the surface of $BaSO_4$ contains $2{\cdot}63 \times 10^{18}$ Ba-ions/g. With $19{\cdot}6 \times 10^{-16}$ cm^2 for the surface area/molecule of $BaSO_4$, we find a surface area of 0·515 m^2/g $BaSO_4$.

We then studied the adsorption of crystal violet (hexamethyl para rosaniline); methylene blue



and picric acid by our precipitates of $BaSO_4$; the adsorption was measured by measuring (with a Cambridge Unicam SP 500 Spectrophotometer) the difference in extinction between the original solution and the solution in which the adsorption equilibrium with a precipitate of $BaSO_4$ had established itself; measurements with crystal violet showed that equilibrium was reached after 30 min shaking; in all our experiments we shook solution and precipitate together for 1 h.

We first studied the extinction as a function of wave length for the 3 substances, and found extinction maxima for

picric acid at	λ = 3550 A
methylene blue at	λ = 6630 A (and λ = 6125 A)
crystal violet at	λ = 5920 A (and λ = 5410 A).

We then established calibration curves, giving the extinction (for each substance at the value of λ given above) as a function of concentration.

We then measured the adsorption from a solution (about 10 cm^3), in which were shaken about 1·7 g of $BaSO_4$; all the solutions had previously been saturated with $BaSO_4$, and all the calibrations were carried out in solutions, containing the dye stuff, and saturated

[1] I. M. KOLTHOFF and W. M. MAC NEVIN, J. Amer. chem. Soc. *59*, 1639 (1937).
[2] L. DE BROUCKÈRE, Ann. Chim. Belg. [10] *19*, 92 (1933).