

Experimental. Methods of bioassay. Secretin activity was determined according to Mutt and Söderberg,¹² cholecystokinin according to Ljungberg.¹³ For the assay of pancreozymin cats operated as for the assay of secretin were used. The pancreas was induced to secrete a juice of low protein content by the injections of secretin, and the protein expelled into the juice in response to injections of pancreozymin was determined.

Materials: Secretin; The pure heptacosapeptide,^{10,11} was used. *Cholecystokinin-pancreozymin;* this was prepared essentially as described in 1961.¹⁴ *Peroxide solution;* 1 ml of a 30 % solution of hydrogen peroxide ("Perhydrol", Merck, Darmstadt, Germany) was made up to 100 ml with 0.05 M AcOH.

Oxidation. Each hormone preparation was dissolved in the peroxide solution to a concentration of 1 mg/ml and allowed to stand at room temperature for 45 min. The solutions were then diluted with five volumes of distilled water and lyophilized. The lyophilized materials were bioassayed. The results are shown in Table 1. It is evident that whereas secretin has lost no activity both cholecystokinin and pancreozymin have been inactivated to the extent of at least 98 %. Under much more drastic conditions secretin too is known to be inactivated by hydrogen peroxide.¹⁵

Reactivation of cholecystokinin-pancreozymin. The inactivated material was dissolved to a concentration of 2 mg/ml in 50 % (w/v) aqueous cysteine hydrochloride. The solutions were stored at room temperature for 72 h in an atmosphere of argon. Appropriate dilutions were then made for the assay. Cysteine in the amounts that contaminated the hormone preparations in these dilutions shows no activity in the assay systems used. The results of the assay are given in Table 1. There was an approximately 90 % recovery of both activities.

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On the Resolution of Absorption Spectra

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An electronic absorption spectrum consists in general of a number of overlapping absorption bands. The simple way of interpreting these spectra, is to determine the different absorption peaks by means of the maxima and inflexions on the spectra. This method does not in any way take care of all the information the spectra can give. It only gives an, in some cases very uncertain, estimate of the wavenumber. Bands that are hidden behind other bands with larger extinction coefficients are not detected at all. If one is interested to study small shifts in absorption spectra for a number of near lying complexes, this spectacular method is very risky since shift of one peak on the spectrum

occurs if the intensity of a near lying peak changes without anything else happening. Determination of extinctions and oscillator strengths will always be very uncertain without any closer analysis of the spectrum.

Recently Challice² has improved the above described method by using an incremental derivative method. Instead of analysing the extinction as a function of wavelength he constructs the second derivative and analyses this curve. The regions of large negative curvature give rise to sharp minima which should correspond to individual band maxima. Even if this method increases the accuracy in the determination of the maxima on the extinction curve, it does not take into account the fact that these maxima do not correspond exactly to the individual band maxima.

The same problem also arises in electron diffraction theory, where the electron diffraction pattern consists of a number of bands of gaussian shape. Since the peaks of the individual bands are used for the determination of the atomic distances in a molecule they should be localized as accurately as possible.

The present author has written an Algol-program which in many cases is able to resolve a spectrum into the individual bands. This program works under the assumption that all bands have a gaussian shape in the frequency scale. As is well known this is not exactly true for absorption corresponding to electronic transition in a molecular complex, even if the deviation from the gaussian shape in most cases is small. If the transition is supposed to occur from the zeroth vibrational state in the electronic ground state it can be shown¹ that the shape is gaussian multiplied with a factor which deviates from one only far from the absorption maximum. This has influenced some investigators¹ to use gaussian curves with different half-widths in the long-wave and the short-wave region. It has been found impossible at the present stage to take these effects into account in the program.

The program works with the least square method. Input data consist of the experimental data and a guess of the result. The program calculates the deviations from these guessed parameters by means

of a linearization process and the calculations continue until the result is self-consistent. The calculation of the deviations is so constructed that when self-consistency occurs the best fit with the experimental curve is obtained.

As an example of the results obtained by this method we can mention the absorption spectrum of Cu(II)-dimethylglyoxime solved in water.³ The experimental extinction curve shows in the investigated region two distinct maxima at 44 250 cm^{-1} and 37 970 cm^{-1} with extinctions of 1.706×10^4 and 1.124×10^4 , respectively. A third region of increased negative curvature occurs at about 28 000 cm^{-1} with an extinction of ca. 0.2×10^4 . Between this region and the top at 37 970 cm^{-1} there is a region where the curve is flattened indicating a fourth small band in this region. This spectrum has been resolved into four individual bands. The result is given in Table 1. The sum of these four

Table 1.

$\nu \text{ cm}^{-1}$		$\epsilon_{\text{max}} \times 10^{-4}$		$\delta_{\frac{1}{2}} \text{ cm}^{-1}$ calc.
obs.	calc.	obs.	calc.	
44 250	44 550	1.706	1.639	3140
37 970	37 520	1.124	1.060	3420
flat region	32 710	—	0.124	1560
~28 000	29 630	~0.2	0.236	3190

absorption bands reproduces the experimental extinction curve within the experimental error, the mean deviation being 0.005×10^4 . It should be noticed that even the largest absorption band has its maximum at the non-negligible distance 300 cm^{-1} from the spectacular value.

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