wovon eines durch nur 21 Peptidbindungen vom «aktiven» Serin getrennt ist. SCHAFFER[3] fand während der Einwirkung von DFP auf Chymotrypsin eine Sofortreaktion mit Tryptophan, der eine sehr langsame mit Tyrosin folgte, die auch bei nicht-enzymatischen Proteinen auftritt. Dadurch wurde Histidin als erste Hälfte des aktiven Zentrums sehr unwahrscheinlich, zumal dessen Stellung bereits durch die Befunde von STEIN et al.[4], BRUICE und SCHMIR[5], ERLANGER[6] und ANFINSEN[3] stark erschüttert war.

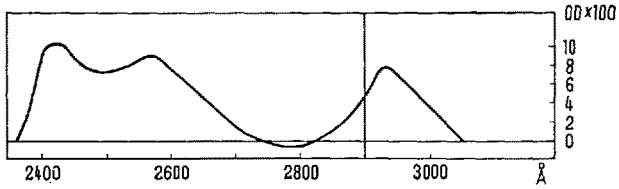

Fig. 1. Differenzspektrum des DIP-Derivates von Chymotrypsin, gegen unbehandeltes Chymotrypsin (33×10^{-6} molar in 8-molarer Harnstofflösung, pH 3,5).

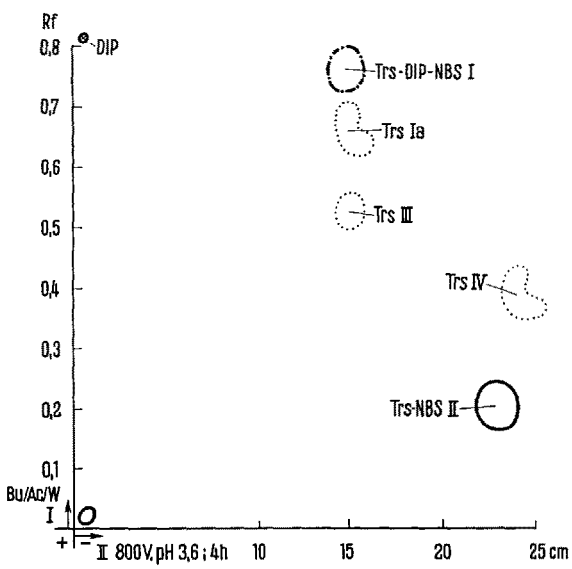

Fig. 2. Elektrochromatogramm von Trypsin: I mit, Ia ohne DIP-Vorbehandlung, I und II ergeben beim DIP-Derivat ausserdem Reaktion auf Phosphor. Ia, III und IV fehlen nach partieller Oxydation des Trypsins mit NBS (Anfärbung mit *p*-Dimethylaminozimtaldehyd).

Wir[7] fanden unter Benutzung des Difference-Spectrophotometers nach CARY an Trypsin und Chymotrypsin nach DFP-Hemmung lediglich eine Verstärkung des für Tryptophan typischen 2. Absorptionsmaximums bei 2910 Å (Figur 1), das nach Inkubation in 8 *M* Harnstoff oder 4 *M* Guanidin noch deutlicher hervortrat und auch nach peptischer Hydrolyse nicht verschwand. Die Oxydation von zwei Tryptophanresten mit N-Bromosuccinimid (NBS) nach PATCHORNIK[8] ergab, dass (1) die spektrale Abweichung erhalten bleibt und (2) nach peptischem Abbau aus dem «Fingerprint» (Elektro-Chromatogramm) hervorgeht, dass NBS beim DIP-Enzym das gleiche Tryptophanylpeptid verschont, das sich um einen kleinen Betrag im Rf-Wert vom korrespondierenden Peptid des nativen Peptides unterscheidet (Figur 2). Die Tryptophanylpeptide wurden auf dem Papier durch Besprühen mit *p*-Dimethylaminozimtaldehyd spezifisch nachgewiesen, mit verdünnter Essigsäure eluiert und auf Phosphor nach FISKE und SUWAROFF geprüft. Ausser in dem durch den Rf-Wert charakterisierten Peptid fand sich eine Spur Phosphat auch bei einem zweiten Tryptophanylpeptid. Phosphatnachweis auf dem ganzen Papier verlief für alle übrigen Peptide negativ. Nach salzsaurer Hydrolyse und unter Berücksichtigung des positiven Try-Nachweises ergab sich folgende Aminosäurenzusammensetzung in stöchiometrischem Verhältnis: Try, Leu, Val, Ser.

Summary. Acid hydrolysis of trypsin and chymotrypsin inhibited by DIP yields seryl-phosphate within a sequence representing one part of the 'active center'. Now it is demonstrated by UV-spectrophotometry that DIP is bound primarily to tryptophane and remains there after peptic degradation. A tetrapeptide containing both tryptophane and phosphate was isolated and analyzed.

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[6] B. F. ERLANGER, Proc. Nat. Acad. Sci. USA *46*, 1430 (1960).
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Anomalous Uptake of Bromsulphthalein by Incubated Liver Slices

Although the hepatic removal of bromsulphthalein (BSP) from the plasma is used clinically as a measure of liver function, the physiological mechanisms involved in this transfer are little understood. Investigation of the isolated uptake phase has been made using incubated liver slices and the results obtained lead BRAUER and PESSOTTI[1] to suggest that in this type of preparation the uptake mechanism was related to binding by intracellular protein. However the measured uptake of about 70% from dilute BSP solutions found with all tissue/solution ratios greater than 5% did not seem in agreement with this conclusion, since it might be expected that tissue/solution ratio would cease to play a significant part only when uptake was close to either 0 or 100%. The experiments described here were carried out to investigate this apparent anomaly, using cat liver slices.

Provided uptake follows a simple adsorption isotherm, it can be shown[2] that at low BSP concentrations the ratio Bound BSP/Total BSP (designated β) is only dependent on the dissociation constant and the concentration of receptor sites, since

[1] R. W. BRAUER and R. L. PESSOTTI, J. Pharmacol. *97*, 358 (1949).
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$$\beta = \frac{\text{Bound BSP}}{\text{Total BSP}} = \frac{1}{1 + \frac{k}{np} + \frac{(D)}{np}} \quad \ldots\ldots \quad (1)$$

where k is the dissociation constant, np the concentration of receptor sites and (D) the molar concentration of BSP present in the medium at *equilibrium*. A method of obtaining β for tissue slices from data obtained from incubation experiments has been described previously together with experimental details applicable to this communication[3].

A graph of the values obtained for β from liver slices incubated in 3 ml of medium plotted against different concentrations of BSP, and the isotherm calculated from equation (1) is shown in Figure 1. It can be seen that β is measurably independent of dye concentrations below 10^{-4} M. As this value is not exceeded in the experiments discussed below, it follows that—if uptake is effected by adsorption—all slices from an homogeneous liver should have the same value for β under standard conditions irrespective of tissue/solution ratio.

Figure 2 shows the values obtained for β plotted against log tissue weight, where the medium volume was 3 ml in each case. It can be seen that at low tissue/solution ratios the values obtained for β were similar, although some random variation occurred, possibly due to the non-homogeneity of the liver or to experimental errors in general. In contrast, the values with high tissue/solution ratios showed a steady decline. Similar results were obtained when the volume of incubation medium was varied and the weight of tissue kept constant.

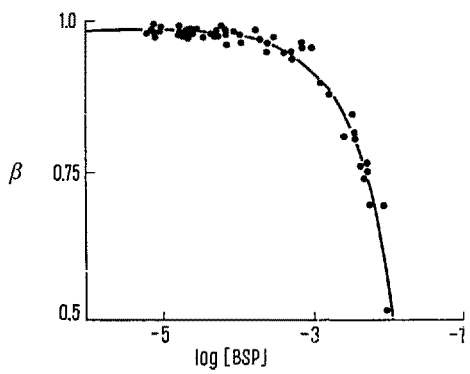

Fig. 1. The experimental values obtained for β plotted against the log of the BSP concentration in the medium at *equilibrium*. The solid line was calculated from $\beta = 1/[1 + k/np + (D)/np]$.

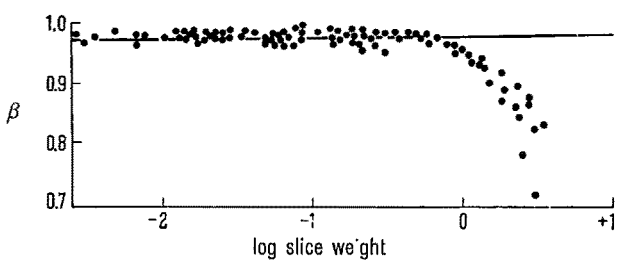

Fig. 2. The experimental values obtained for β plotted against the log of the weight of liver tissue used. The medium volume was 3 ml in each case.

The diffusion of soluble protein from slice to medium during incubation, occurring despite an initial washing, seemed the most probable cause of these anomalous values. The reduced protein content of the liver slice probably produced a small but constant error in the values calculated for β[4], but this free protein present in the medium must bind and retain BSP and thus introduce a further error in the calculation of β, the magnitude of which might be excepted to depend upon the tissue/solution ratio. Theoretical calculations indicate that the deviations shown in Figure 2 may be satisfactorily accounted for in this way, and some experimental confirmation has been obtained by protein analysis of the post-incubation medium (by the method of TOMBS, SOUTER, and MACLAGAN[5]) and by the values of β obtained with denatured slices and also by repeated incubations of the same slice. Thus: (a) the protein content of the medium after the incubation experiments was found to be very small at tissue/solution ratios which produced a constant value for β, but showed a rapid and apparently linear increase over the range in which a decline was seen in the value of β; (b) heat denaturation, effected by immersing the slices in saline solution at 80°C for 5 min immediately prior to incubation, might be expected to reduce the diffusion of soluble protein into the medium. At high tissue/solution ratios only a slight deviation from the expected value for β was observed; (c) the value of β obtained with large slices was found to rise to the expected value with successive incubations and to be accompanied by a fall in the concentration of free protein in the medium.

These experiments indicate that uptake of BSP by liver slices during incubation follows a simple adsorption isotherm in relation to both dye concentration and tissue/solution ratio. It would appear that anomalous values obtained with high tissue/solution ratios may be artifacts due to the binding of BSP soluble proteins which diffuse into the medium during incubation[6].

Résumé. La quantité de BSP prise par des coupes de foie durant l'incubation suit une simple courbe isothermique d'adsorption.

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[6] The author gratefully acknowledges support in the form of research awards from C. L. Herman and the M. G. Fourcade bequests.