lund and Brattsten [1]. The fractionation was carried out by continuous zone electrophoresis [2] in a starch supporting medium at pH = 8.5 and the ionic strength = 0.03. The presence of acute phase protein in the fractions was determined by the capsular swelling reaction (Löfström [3]). Pneumococci suspensions of type 23 B were used. Reactive substance was found in the γ-globulin fractions exclusively, the capsular swelling titration curve showing a rather striking conformity with the γ-globulin distribution curve.

In order to check these results the same fractions were afterwards analysed against a pneumococci suspension of type 27, which was even more sensitive than type 23 B, and by precipitation with C-reactive protein antiserum (obtained from Schieffelin & Co., New York). Both tests confirmed the findings reported.

In the fractions obtained in the electrophoretic separation the reactive substance was present in mixture with other proteins of the γ-globulin type. This material has been studied as to its solubility in the presence of ammonium sulfate. As judged from the capsular swelling analyses, the reactive substance remains in solution at half saturation of this salt, but some precipitation occurs as the salt content is further increased. However, complete precipitation could not be achieved this way; even at saturation of ammonium sulfate the supernate showed a relatively strong activity reaction.

The initial fractionation experiment also showed that adequate isolation of acute phase protein could be obtained at a considerably lower total resolution than that used. Hence the procedure could be simplified by increasing the rate of fractionation. In subsequent work a quantity corresponding to 1.5 ml of undiluted serum has been fractionated per hour. In all, 65 ml of serum has been worked up. This includes material from three different cases of pneumonia in the acute stage. In order to establish a possible influence of the ionic strength on the mobility of the active material two fractionations have also been carried out at the ionic strength 0.06, other conditions remaining unchanged. After enrichment of the proteins, the fractions have been analysed by the capsular swelling reaction using type 23 B and 27 pneumococci suspensions and the C-reactive protein antiserum test. In all instances complete qualitative agreement with the primary result was obtained, thus showing that acute phase protein, developed in the diseases stated, resembles the γ-globulins in electrophoresis under the reported conditions.

The capsular swelling reaction was found to be more sensitive than the antiserum reaction.

Six months of storage in the frozen state (at —10° C, in Ringer's solution) did not affect the titer of the active substance.

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On the Localization and Composition of Phosphoprotein in *Esherichia coli B*

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In an earlier communication the preparation of a new phosphorylated amino acid in *Lactobacillus casei* has been reported [1,2]. Several peptides containing this compound were also found. In attempts to prepare more high molecular phosphoproteins the following experiments were carried out with *E. coli B*.

The bacteria was cultivated on Friedleins synthetic medium containing Na-lactate as the carbon source and NH_4Cl as nitrogen source [3]. To this medium was added 1 mC radioactive phosphate per liter. Procedures for cultivation and separation of bacteria have been described by Palmstierna and Holme [4,5]. In this experiment the initial bacterial density was 1.25×10^9 cells/ml. At the end after 120 minutes of incubation the density was 1.95×10^9 cells/ml. 24 g of freeze-dried bacteria was obtained.

Suspensions containing 0.5 g dry weight bacteria/20 ml in 0.04 M Tris-buffer were shaken in the Raytheon disintegrator for 30 minutes during continous cooling. The cell walls possibly contaminated with some cytoplasmatic debris [6] were removed from the lysed solution by centrifugation at 40 000 r.p.m. One part of

Table 1. R_G-values of the two ninhydrin-reacting substances from fraction 3 and 5 of the cell wall after complete hydrolysis. Run in different organic solvents on Munktell OB filter paper. The corresponding values for the hydrolyzed amino acid from L. casei are included.

$$R_G \text{ value} = \frac{R_F \text{ value of unknown substance}}{R_F \text{ value of glycine}}$$

	E. coli		*L. casei*
	Fraction 3	Fraction 5	
Phenol	0.41	0.95	1.26
Pyridine-water	1.77	0.95	1.96
n-Butanol-HAc-water	0.80	1.10	1.81
*Iso*butyric acid-NH_3-water	0.90	1.85	1.00
Pyridine-*iso*amylalcohol-water	1.10	1.50	2.11

centrifugate was fractionated at low temperature with ethanol and Zn-acetate mainly according to Cohn [7] and the other part with acetone [8] and Zn-acetate. From the five fractions of the ethanol and acetone precipitations and the cell wall the Schneider protein was prepared as previously described [1,9]. The partial acid hydrolysates were chromatographed on Dowex 50 columns.

The peaks containing ninhydrin-positive material were analyzed for activity. About 80 % of the total activity was present in the 5 peaks of the cell wall hydrolysate while the remaining 20 % were rather evenly distributed on the 17 peaks of the ethanol and acetone hydrolysates (Table 2). 20 % by weight of the dried bacteria was recovered as cell walls.

After complete hydrolysis all fractions were run on one dimensional chromatogram. The material from the two main peaks of the cell wall hydrolysate (3 and 5) gave one major spot with only traces of other ninhydrin-positive compounds. Table 1 shows the positions of these spots in several solvent systems. It seems probable that these two substances differ from the phosphorylated amino acid in *L. casei*. Furthermore on paper electrophoresis the two new substances behaved as more basic amino acids. With the copper complex method [10] the most basic material reacted like a non α-amino acid. The two new phosphorylated compounds were also present in the ethanol and acetone fractions of the bacterial cytoplasmic material.

Table 2.

Fractions numbered as leaving the Dowex 50 Column	Specific activity in cpm/μg P					
	Cell walls	50 % ethanol	Zn-acetate in 80 % ethanol	40 % acetone	60 % acetone	Zn-acetate in 60 % acetone
1	1 022	1 013	1 030	518	736	—
2	933	835	855	386	640	—
3	1 126	778	451	755	565	—
4	545	496	—	735	405	—
5	658	—	—	455	—	—
6	—	—	—	756	—	—

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 [1], *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 3 130 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 absorbtion 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 frequences or dissociation.

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

Thus, the formula of Perrin [2] 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-absorbtion 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 absorbtion. 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 absorbtion 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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