Fig. 1.

sponding to the position of a synthetic specimen of phosphoserine was observed. Serine was completely set free by hydrolysis with 2 *N* HCl at 120° for 24 hours. When large samples of the hydrolysate (1 mg) were analyzed chromatographically, a very faint alanine spot was observed in addition to the large serine spot. At present it is not possible to decide whether the alanine was present as part of a phosphoserine peptide in the crystalline preparation, or formed from phosphoserine during hydrolysis. The phosphopeptides left in the residue after crystallization of phosphoserine are being investigated with the interest focused primarily on the question of whether or not phosphothreonine is present in the mixture.

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On Homospecific Liver Pyrophosphatases

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Recent investigations[1] have demonstrated the existence of four isodynamic pyrophosphatases in rat liver. Swanson [2], however, found only two pyrophosphatases in rat liver. It therefore seems of interest to report some further studies on these enzymes in rat liver and in liver of other species. At the same time a correction regarding the magnesium ion concentration in the previous experiments [1] is given.

The livers of freshly killed animals were used throughout. The assay of pyrophosphatase activity was performed at 37° C as previously described [1]. At pH values below 6 the "third method" was used, *i. e.* the inorganic phosphate liberated was isolated as hydroxyl apatite before estimation. At higher pH values the phosphorus was determined directly on an aliquot of the protein-free filtrate obtained according to the "second method". The pyrophosphate concentration in the enzym digestion mixture was kept at 10^{-3} *M* in all experiments.

For the acid range up to pH 5.8 acetate buffers were used, from pH 6 to 7.6 cacodylate buffers (Plumel [3]) succesfully replaced maleic acid and collidine buffers. Over pH 7.6 ammonium chloride-ammonia buffers were used.

The activating reagent was prepared from a stock solution of molar $MgSO_4$ by dilution with 0.05 *M* buffer. When a new stock solution was prepared (autumn 1950) the enzyme values were lower than before. A check-up study showed that the Mg ion concentration giving maximum activation was about 0.01 *M* instead of 0.2 *M*. As the earlier studies were clearly made with optimal activation con-

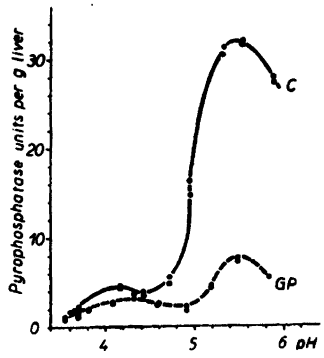

Fig. 1. Acid pyrophosphatases in liver. Dots and fully drawn line: cat liver. Circles and dotted line: guinea pig.

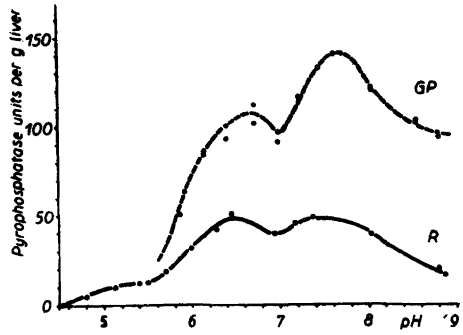

Fig. 2. Pyrophosphatases in liver. Dots and fully drawn line: rabbit liver. Circles and dotted line: guinea pig.

centration the old stock solution which was prepared by a technician, and which I had not controlled, apparently was only one twentieth of the intended concentration. With the proper correction my earlier results should be comparable with other investigations, however.

The pH-activity curves prepared under the conditions described show two peaks in the acid region at about 4.2 and 5.5, as is evident from Fig. 1. With hamster and rabbit liver there is a plateau at pH 5 to 5.5. The curve then rises steeply to the maximum of the neutral pyrophosphatase. In several experiments with rat and guinea pig liver the pH 5 enzyme is indicated by a hump in the curve only. The pH 5 pyrophosphatase may therefore easily be overlooked.

With collidine (method 3) and with cacodylate as buffers the neutral pyrophosphatase has its maximum at pH 6.6 to 7.0. On the alkaline side the optimum appears between pH 7.6 and 8 (Fig. 2). These two pyrophosphatases are some ten times more active than the acid homospecific enzymes with activities from 50 to more than 300 units per g liver (1 unit = 1 micromol of phosphorus liberated per minute in the test system).

Distinction of the homospecific pyrophosphatases by other properties than the pH optima is possible, *e. g.* through selective inactivation [4] or inhibition [1]. The pyrophosphatase pH 4 is inhibited by 0.01 *M* Mg^{++} from 10 to 54 % in guinea pig liver. The pH 5 pyrophosphatase, on the other

Table 1. Pyrophosphatase activities before and after acetone precipitation. Expt. 143 aqueous homogenate pH 6.88. Expt. 193 homogenate in 0.2 % ammonia and 0.003 M $MgCl_2$. Expt. 223 homogenate in 0.2 % ammonia pH 9.2.

	I. Activity at pH 5.2			II. Activity at pH 6.8			Activity ratio I/II		
Expt. no.	143	193	223	143	193	223	143	193	223
Original homogenate	22.0	18.6	20.2	100	99	67.7	0.22	0.188	0.298
40—75 % acetone ppt	8.41	4.45	0.26	23.9	65.6	3.75	0.35	0.068	0.07

hand, is activated considerably, the increase in activity amounting to about 200 %. As the neutral pyrophosphatase is also strongly activated by magnesium it seems essential to demonstrate the mutual independence of the pyrophosphatase 5 and the neutral one. This was tried on rat liver homogenates by fractional precipitation with acetone at 0° C. Comparison between the original activities and the activities of the proteins precipitated by 40 to 75 % acetone demonstrates the requested independence as appears from Table 1. The activity ratio may be changed in opposite directions by slight changes in pH etc. This means that the two enzymatic activities are differently affected, *i. e.* a selective partial inactivation occurs.

This is further evidence for the existence of an acid pyrophosphatase with optimum at pH 5 – 5.5 in addition to the three earlier known isodynamic enzymes. The enzyme seems to be present not only in rat liver but also in the liver of guinea pig, hamster, rabbit and cat.

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New Salts of Benzylpenicillin with Organic Bases

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It is known that benzylpenicillin forms crystalline well defined salts with several organic bases, and salts with secondary [1] and tertiary [1–4] as well as primary [1,5–7] amines have been described.

In an attempt to find bases, which might be useful for the isolation of penicillin, a further series of organic amines have been investigated. The penicillin salts were all prepared in essentially the same way; namely, by the addition of a slight excess of the base, dissolved in acetone to a solution of pure free benzylpenicillin in amyl acetate-acetone. Some of the salts separated in crystalline form, others as oils, as shown in the following table.

Base	Reaction product with benzylpenicillin
N-Monomethylethylenediamine [8]	oil
N-Monoethylethylenediamine [8]	»
2-Methylimidazoline [9]	crystals
2-Ethylimidazoline [9]	oil
2-Propylimidazoline [9]	crystals
2-Amylimidazoline [9]	oil
2-Benzylimidazoline	»
2,4(5)-Dimethylimidazoline	»
2-Ethyl-4(5)-methylimidazoline	crystals
2-Propyl-4(5)-methylimidazoline	»

The preparation and properties of the dialkylated imidazolines referred to will be described in a forthcoming communication in this Journal.

Only one example of the preparation of the benzylpenicillin salts will be given in detail.

The 2-propylimidazoline salt of benzylpenicillin. 5.0 g of sodium benzylpenicillin was dissolved in 20 ml of water at 0 – 5° C, 40 ml of cold amyl acetate was added, and then, with vigorous shaking, 4.5 ml of 60 % phosphoric acid. The aqueous layer was separated and extracted with an additional 10-ml portion of cold amyl acetate. The combined