exception of compounds Id and Ig (desacetyl-rifampicin) which show higher activity and Ie and If which maintain their activity. All the compounds are currently being evaluated for the in vivo activity and toxicity. The results will be published elsewhere.

In conclusion, it must be pointed out that the new class of rifamycins described above proves the possibility of modifying the antibacterial spectrum of rifamycins by a limited chemical manipulation of the aliphatic bridge of the molecule[12]. This finding constitutes an additional evidence of the versatility of the molecule, giving a further contribution to better knowledge of the structure-activity relationship for rifamycins[13].

Riassunto. Vengono descritte la sintesi, le proprietà chimico-fisiche e l'attività antibatterica in vitro di alcune desacetil-rifamicine. Vengono discusse le variazioni osservate nello spettro antibatterico in confronto con gli analoghi acetilati al C-25.

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Molecular Properties of Pepsin as Studied by Gel Filtration

According to Golub and Picket[1] pepsin presents, by varying the pH, molecular disorganization. By using ultracentrifugation, Dieu was able to show[2] that below pH 5.5 and between pH 7.0 and 10.0, pepsin is formed by a homogeneous molecule, while between pH 6.25 and 7.0, it appears in more than one fraction. This finding would be explained as a consequence of the existence of the native and the denatured forms of the enzyme. The same author observed that denatured pepsin undergoes an enlargement of the molecule with respect to the active form. Edelhoch[3,4] has also showed that the molecule of alkali denatured pepsin presents a dilatation probably due to a deformation of the helix of the natural molecule.

By following denaturation with alkali, temperature and iodination, Ishii and Yasuda[5] found that the value of osmotic pressure increases with respect to the active enzyme. Conformational changes of pepsin molecule above pH 5.0 were also observed by Blumenfeld et al.[6].

Recently, phenomena of polymerization and conformational changes of several enzymes have been studied by gel filtration[7–9]. It therefore seemed of interest to investigate the inactivation process of pepsin and the accompanying changes of the molecule structure, by using gel filtration.

To this end, a series of experiments were performed, in which equal amounts of pepsin were fractionated at various pHs, ranging from 6.5–7.25. Variations of the elution patterns of the protein profiles and of the enzymatic activity were taken into consideration.

For all the experiments × 2 crystallized pepsin from 'Sigma' Chemical Co. was used; gel filtration was performed on G-100 and G-75 Sephadex column (1.8 × 90 cm) in a cold room. *Tris*-phosphate buffers (0.005 *M Tris* with added H_3PO_4 to desired pH value) containing 0.15 *M* NaCl, were used. The pepsin samples were prepared by dissolving 20 mg of enzyme in 4 ml of the same buffer used to equilibrate and elute the Sephadex column. The pH measurements were done with a 'Radiometer' type TT-1. Proteins were assayed by the Lowry[10] method using bovine albumin 'Sigma' Chemical Co. as standard.

The proteolytic activity of pepsin was assayed in each fraction by using hemoglobin as substrate, in a 0.2 *M* HCl-KCl buffer pH 1.8. The hydrolysis products, separated with 5% TCA and centrifugation, were read at 280 nm.

Typical patterns of gel filtration on Sephadex G-100 at various pHs, are reported in Figure 1. It appears that pepsin, at pHs below 6.75, is localized in one symmetrical peak and still retain high enzymatic activity. At pH 6.75, the protein peak begins to appear deformed in its ascendent part which shows a net shoulder. The elution volume is decreased and consequently an increase of the molecular weight is noted. The enzymatic activity appears now strongly reduced and it is localized only in the fractions corresponding to the original, lower molecular form of the enzyme. At pH 6.85, the elution profile shows 2 distinct protein peaks; the first one is completely devoid of enzymatic activity, while the second peak still maintains a residual activity.

Increasing the pH from 6.85 to 7.25, the conversion process of the active form into the larger, inactive, molecular form is practically completed (Figure 1 D, E, F). The molecular weight of this new form of pepsin, appearing at pH 7.25, was estimated by comparing its elution volume with that of crystallized bovine albumin.

As shown in Figure 2, both alkali denatured pepsin and albumin had approximately the same elution volume and therefore appeared to have similar molecular size.

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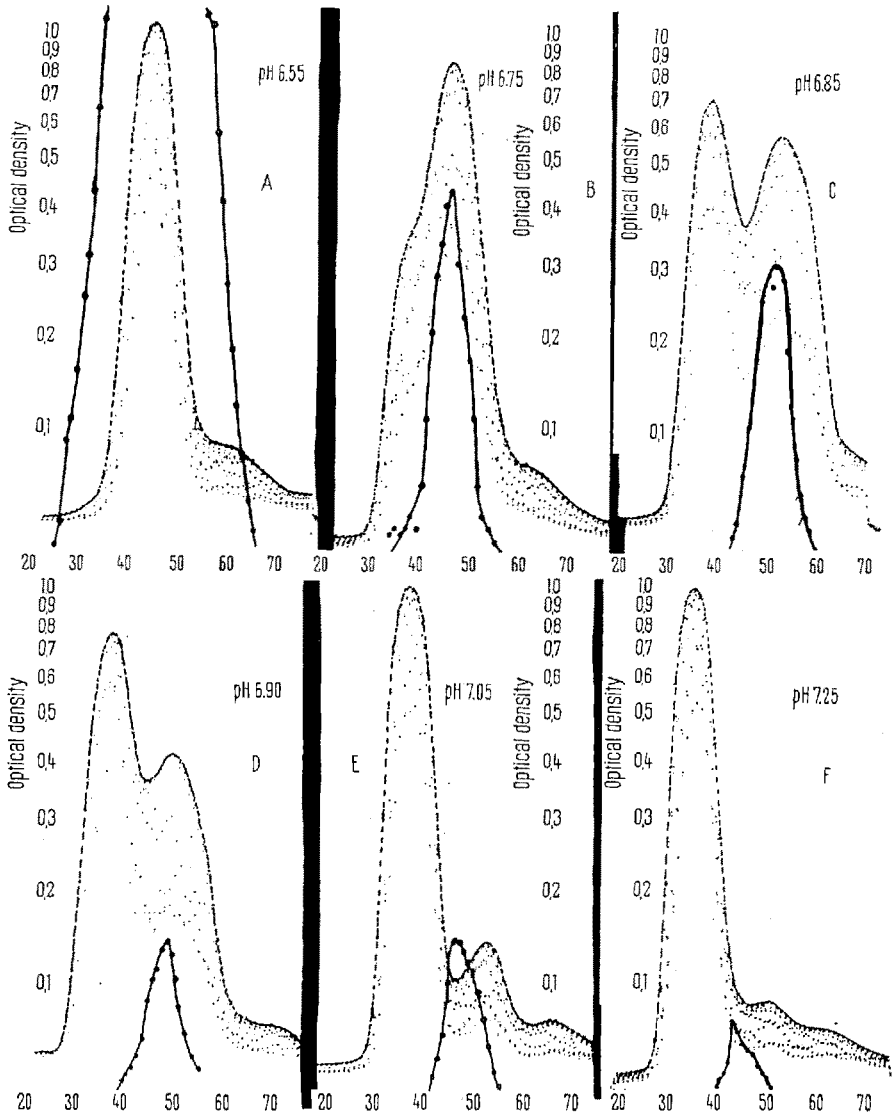

Fig. 1. Gel-filtration on Sephadex G-100 of pepsin, at various pHs: dotted line, protein concentration; solid line, proteolytic activity on hemoglobin as substrate.

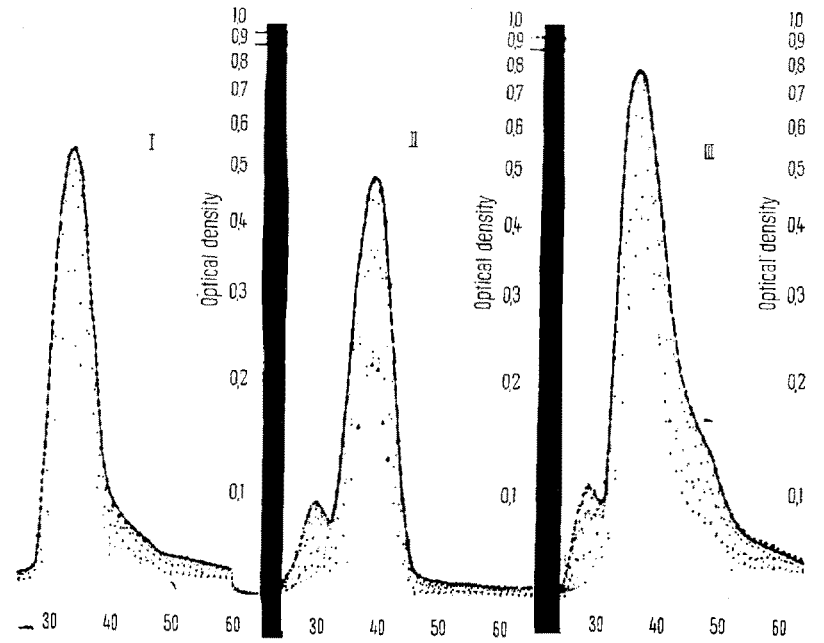

Fig. 2. Gel-filtration patterns of I pepsin, II bovine albumin and III pepsin + albumin, on the same Sephadex G-100 column, equilibrated with *Tris*-phosphate buffer at pH 7.25.

Since the molecular weight of the active pepsin (below pH 6.0) is about 34,000, it would appear that the inactivation process which occurs between pH 6.75 and 7.25, is accompanied by a dimerization of the active subunit.

From the experiments reported above, it appears that the loss of the catalytic property of the enzyme parallels the gradual conversion of the active monomer into an inactive aggregate. Further analysis of this process is now in progress by using sucrose density gradients centrifugation and disc-electrophoresis[11].

Riassunto. A bassa temperatura (5 °C) variando il pH del mezzo da 6,50–7,25, la pepsina varia le sue dimensioni molecolari e si inattiva. Tale fenomeno, evidenziato per gel filtrazione, sembra sia dovuto alla formazione di un aggregato della pepsina di peso molecolare molto vicino a quello dell'albumina bovina.

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Metabolism of Alcohol in Partially Hepatectomized Rats Exposed to Cold

Several workers have shown that cold exposure in rats resulted in an increased oxidative activity of the liver[1–6]. WEISS and MOSS[5] reported that the liver, an organ which has one of the lowest metabolic rates among several tissues, showed the greatest metabolic increase in response to cold. The latter authors also have shown that the metabolic rate of liver slices in partially hepatectomized rats increased 20% when animals are kept at room temperature and slightly over twice this amount when exposed to a cold environment.

Earlier we reported that rats exposed to cold, metabolized alcohol faster than controls kept at room temperature[7]. In the present experiment, we have determined the effect of partial hepatectomy on the ability of cold exposed and room temperature housed rats to metabolize alcohol[8].

Methods. Male albino rats of Sprague-Dawley strain weighing 190–210 g were divided into 6 groups of 10 rats/group. The first 3 groups consisted of partially hepatectomized, sham-operated, and non-operated control animals which were placed in a cold room at a temperature of − 5 °C, 48 h after surgical intervention where they remained for a period of 5 days. The other 3 groups, divided and operated upon as above were kept at room temperature (20° ± 2 °C) for 7 days. On the eighth post-operative day each animal received i.p. 0.8 g/kg of uniformly labelled ^{14}C-alcohol (specific activity 0.23 μC/mM of alcohol) as a 20% aqueous alcohol solution. Respiratory $^{14}CO_2$ was collected by the method described previously[7]. Partial hepatectomy was performed as described by HIGGINS and ANDERSON[9].

Analysis of variance was performed with an IBM computer, Model 650, according to the 'R × 2 Tables' of YATES[10].

Results. The most striking difference in $^{14}CO_2$ recovery occurred during the first 3 h following alcohol administration (Table I). During this time, all animals exposed to cold metabolized as much alcohol as the room temperature-housed rats oxidized in 9 h. The effect of temperature on the recovery of labelled carbon dioxide was

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Table I. Mean values of cumulative recoveries of exhaled $^{14}CO_2$ (mM) from metabolized alcohol

Treatment	Interval of CO_2 collection				
	1 h	3 h	5 h	7 h	9 h
Control, room temperature	0.71	3.60	4.42	4.70	4.86
Control, cold	1.30	4.83	5.32	5.49	5.57
Sham, room temperature	0.78	3.52	4.50	4.80	4.95
Sham, cold	1.36	4.84	5.44	5.64	5.73
Partial hepatectomy, room temperature	0.63	3.23	4.30	4.58	4.73
Partial hepatectomy, cold	1.33	4.70	5.16	5.39	5.47
F of effect of temperature	31.98[a]	39.37[a]	35.09[a]	31.09[a]	28.48[a]
F of treatment	0.26	0.53	0.55	0.85	1.02

F of effect of treatment = 9.18[a]; F of effect of temperature = 0.37. [a] $P < 0.01$.