

TABLE I  
RECOVERY OF N-2,4-DINITROPHENYLHYDRAZIDES OF LOWER FATTY ACIDS  
FROM THE CHROMATOGRAPHIC COLUMN

| <i>N-2,4-Dinitrophenylhydrazide</i> | <i>Added<br/>(<math>\mu</math>g)</i> | <i>Recovered<br/>(<math>\mu</math>g)</i> | <i>Recovery<br/>(%)</i> |
|-------------------------------------|--------------------------------------|--|-------------------------|
| Acetic acid                         | 160                                  | 168                                      | 105                     |
| Propionic acid                      | 305                                  | 320                                      | 105                     |
| Isobutyric acid                     | 176                                  | 173                                      | 98                      |
| <i>n</i> -Butyric acid              | 269                                  | 272                                      | 101                     |
| Trimethylacetic acid                | 204                                  | 197                                      | 97                      |
| $\alpha$ -Methylbutyric acid        | 315                                  | 330                                      | 105                     |
| Isovaleric acid                     | 461                                  | 482                                      | 105                     |
| <i>n</i> -Valeric acid              | 390                                  | 403                                      | 103                     |
| Caproic acid                        | 441                                  | 446                                      | 101                     |

The elution sequence was similar to that of the free fatty acids from a column of Amberlite IRC 50, but the efficiency was increased, that is the two isomers of butyric acid and the four isomers of pentanoic acid were separated as their N-2,4-dinitrophenylhydrazides.

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### Chromatographic detection of sugars of growing cartilage

In recent years, the intense metabolic activity of growing cartilage has been extensively investigated<sup>1</sup>; a qualitative and quantitative study of the composition of this tissue seems therefore highly desirable. In the present paper a report is presented of a qualitative examination of the sugar content of growing cartilage.

Investigations were carried out on 40 days old albino rats and on 50 days old rabbits. Cartilage samples were cut out from the proximal end of the "tibia", carefully washed with water and hydrolysed for 10 h in *N*/10 HCl at 100°; 1 ml of acid solution was used per 10 mg of cartilage. The hydrolysate was filtered and evaporated to dryness under vacuum at 50°. The dry residue was repeatedly dissolved in a small amount of water and evaporated to dryness until the pH of the solution was about 5. The residue was then dissolved in a small volume of distilled water and applied to the

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top of a column (450 × 26 mm) packed with a mixture of equal parts of IMAC A 17 and IMAC C 100 resins (both of Montecatini S.p.A., Milan). The first is a weakly anionic, the second a weakly cationic exchanger. Both were of 80–160 mesh.

The extract was washed out of the column with 150 ml of water at the rate of 0.8 ml/min. The effluent, omitting the dead volume, was collected in a container containing 1–2 ml toluene and evaporated to dryness under vacuum at 50°. The

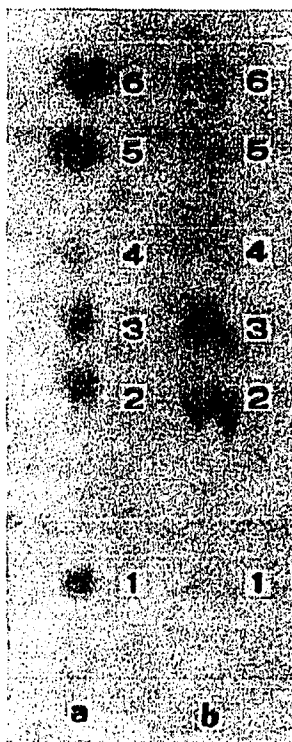


Fig. 1. Paper chromatography of sugars of growing cartilage. Solvent: *n*-butanol–pyridine–benzene–water (5:3:1:3)<sup>2</sup>. Details of the procedure are reported in the text. a = reference mixture of pure sugars; b = cartilage hydrolysate. (1) lactose, (2) galactose, (3) glucose, (4) fructose, (5) xylose, (6) ribose.

residue was dissolved in 0.5 ml of water saturated with benzoic acid. In order to obtain a complete recovery of the extract, the walls of the vessel were repeatedly washed with hot methanol. The aqueous solution and the methanol washings were united and evaporated to about 0.5 ml under a current of dry air.

0.2 ml of the concentrated fluid (corresponding to about 100 mg of fresh tissue) was chromatographed on Whatman No. 1 paper using *n*-butanol–pyridine–benzene–water (5:3:1:3) as solvent according to WHITE AND HESS<sup>2</sup>, and the sugar spots were detected by spraying the chromatogram with a mixture (1:1, v/v) of a 2% solution of double distilled aniline in ethanol and 0.2 *M* citric acid<sup>2</sup>. After drying, the paper was kept for a few minutes at 100°.

A typical chromatogram is reproduced in Fig. 1.

The identification of the sugars was carried out by comparison with a known mixture of chemically pure sugars (see Fig. 1) and by separate addition of pure samples of each of the sugars to the extract.

Identical results were obtained with rats and rabbits. As evident from the described procedure, these results concern total sugar of growing cartilage, *i.e.* free sugars and sugars arising from acid hydrolysis of the polysaccharides.

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#### BOOK REVIEWS

*Comprehensive Analytical Chemistry*, Volume IA, *Classical Analysis*, edited by CECIL L. WILSON AND DAVID W. WILSON, Elsevier Publishing Company, Amsterdam, 1959, xx + 577 pages, price £ 5.5.0.

The success of RODD'S "*Chemistry of Carbon Compounds*", which is one of the best texts on organic chemistry, has induced the publishers to promote another very important comprehensive work, this time in the field of analytical chemistry, *viz.* "*Comprehensive Analytical Chemistry*", edited by CECIL WILSON AND DAVID WILSON.

The first volume of this work deals with classical analysis and part IA of this volume, which appeared recently, comprises six chapters, the first a general introduction, the others devoted to analytical processes, gas analysis, inorganic qualitative analysis, organic qualitative analysis, inorganic gravimetric analysis.

It must be recognized that the task undertaken by the Editors is very difficult owing to the vastness of this work.

A first observation must be made: "*Comprehensive Analytical Chemistry*" should be regarded more as a source of information and literature rather than as a book to be used in the laboratory when performing an analysis in practice.

Only with this in mind can it be understood why, for instance, only two pages are dedicated to countercurrent distribution.

The authors of the different sections have certainly been well chosen, but notwithstanding this the sections do not all appear to be of the same high standard, *e.g.* the treatment of qualitative organic analysis is rather inadequate, no mention being made of systematic methods; in the case of inorganic qualitative analysis it is a pity that the systematic classical separation (according to TREADWELL) has not been mentioned at all, although this volume is specifically devoted to classical analysis. Apart from these shortcomings, understandable owing to the huge task of the Editors,

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