Intestinal Absorption of Glucosamine and N-Acetylglucosamine

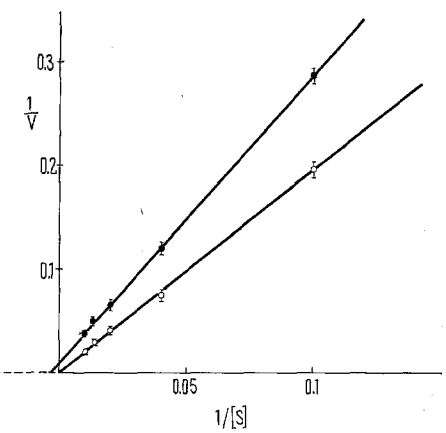
This work is intended as a study of the intestinal absorption of glucosamine and GlcNAc (N-acetylglucosamine) to establish whether these compounds are absorbed with or without change in their chemical structure, by means of active transport and with or without the intervention of a carrier.

For our studies, male rats of the Wistar stock of the average weight of 200 g were used. In general, two experimental conditions were followed: A) Sacs of everted small intestine, prepared according to the indications of WILSON and WISEMAN[1], were incubated in 5 ml of KREBS-HENSELEIT[2] bicarbonate buffer containing 10 m*M* glucosamine or GlcNAc. When buffers without Na^+ were used, *Tris*-HCl buffer (pH 7.4) was substituted for NaCl in equimolar fashion[3]. B) Transversely cut segments (strips) were prepared according to CRANE and MANDELSTAM[4]. Four segments, chosen according to the indications of ROSENBERG et al.[3], were incubated in 2 ml of a solution as reported by SCHULTZ and YU-TU[5]. The total osmolarity of the solution used, which also contained glucosamine or GlcNAc plus *Tris*-HCl (pH 7.4), was 320 milliosmols. When amino acids, sugars or amino sugars were added to the buffers, the concentration of *Tris*-HCl was lowered so that the total osmolarity of the solution remained unchanged. All the solutions used, in all cases, contained also glucosamine $1^{14}C$ or GlcNAc $1^{14}C$ (10.000 counts/min per ml). The GlcNAc $1^{14}C$ was chemically synthesized by following the indications previously reported[6].

Everted sacs, filled with 1 ml of the incubation medium, were incubated for 15, 30 or 60 min. The transmural absorption of the glucosamine was evaluated as the concentration ratio (S/M) between the serosal (S) and the mucosal compartment (M). In the experiments using strips, these, after 10 min of preincubation in a medium without amino sugars, were rapidly transferred into a new medium containing the additives suitable for each experiment and then incubated for 15,30 or 60 min. At the end of the incubation period, the segments were taken out and excess incubation medium removed by pressing them against the sides of a glass beaker. The strips were then weighed and rinsed in a medium identical to that of the incubation but without glucosamine $1^{14}C$ or GlcNAc $1^{14}C$. The segments were then introduced into tubes containing 2 ml H_2O and these were placed in a water-bath at 100°C for 10 min and centrifuged at 1000 rpm for 5 min[7]. Tissue accumulation of the (^{14}C) amino sugars was expressed as a distribution ratio or

$$\frac{\text{counts/min per ml intracellular fluid (ICF)}}{\text{counts/min per ml medium (ECF)}}.$$

Net tissue counts were corrected for extracellular space values, calculated according to the indications of ROSENBERG et al.[8]. Total tissue water was determined by the difference between wet tissue weight and tissue weight after drying at 110°C for 8 h. All the incubations were carried out in a Dubnoff metabolic shaker at 37°C in an atmosphere of O_2–CO_2 (95:5 v/v). Radioactivity was measured with a liquid scintillation counter with an efficiency of 81% for (^{14}C), using 10 ml of a solution composed of PPO (4 g/l); POPOP (100 mg/l) in toluene-metylcellosolve solution (1:1 v/v). The channel ratio method was used for determination of efficiency. The experiments carried out both on everted sacs and on intestinal segments agree in indicating that in the small intestine of the rat there is no active accumulation of glucosamine and GlcNAc. In fact the distribution ratio never showed values superior to 1. On the other hand, both strips and sacs of everted small intestine are able, under standard conditions, to effect concentration against a gradient using glucose as a substrate. Furthermore, the addition of 0.5 or 1 m*M* of phlorizin, the substitution of Na^+ with $Tris^+$, the substitution of the gaseous mixture O_2–CO_2 (95:5 v/v) with air or N_2, caused no perceptible variations in the distribution ratio. The influx of glucosamine and of GlcNAc studied with segments of rat's intestines, in the absence of Na^+, is plotted in the Figure as a function of the concentration of glucosamine or of GlcNAc in the mucosal solution. For glucosamine, a Lineweaver-Burk plot of these data reaches the ordinate at a point which is significantly different from zero; this suggestes that the sugar in question penetrates the intestinal cells by a saturable process with a definite Vmax. On the contrary, the Lineweaver-Burk plot drawn for GlcNAc (Figure) shows a line, the intercept of which does not differ significantly from the original. Therefore it was not possible to define a Vmax for GlcNAc. We repeated the experiment, shown on the Figure, 12 times. In all cases the data seem to indicate that the transport of glucosamine into the intestinal cells is carrier mediated and inde-



Effect of concentration on transport rate, plotted according to Lineweaver and Burk, for glucosamine (●) and N-acetylglucosamine (○). Each point is the average of 12 determinations ± S.E. S, the initial medium concentration (m*M*); V, the final tissue concentration (mmoles/l intracellular water). Strips were incubated for 15 min as described in text. Transport constants were derived as follows: $K_t = I/x$ and Vmax = I/y . x and y are means of 12 experiments. Glucosamine intercept on I/S axis: x, 3×10^{-3}; S.E., $\pm\ 0.17\times10^{-3}$; 99% confidence limits = $\pm\ 0.52\times10^{-3}$. Intercept on I/V axis: y, 8.5×10^{-3}; S.E., $\pm\ 0.68\times10^{-3}$; 99% confidence limits = $\pm\ 2.10\times10^{-3}$. $K_t = 1/3\times10^{-3} = 333$ m*M*; Vmax = $1/8.5\times10^{-3} = 117$ m*M*. N-acetylglucosamine: x and y are not significantly different from zero. 'Confidence limits' were obtained according to JERVIS and SMYTH[10].

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[5] S. G. SCHULTZ and L. YU-TU, Biochim. biophys. Acta *196*, 351 (1970).
[6] G. TESORIERE, R. VENTO, D. MAGISTRO and F. DONES, Ital. J. Biochem. *19*, 139 (1970).
[7] R. M. PRATT JR. and C. TERNER, Biochim. biophys. Acta *225*, 113 (1971).
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pendent of Na^+; on the other hand, the influx of GlcNAc appears to be the expression of a simple process of diffusion.

Finally, in certain experiments in which everted sacs were used, an attempt was made to find out whether the absorption of glucosamine and GlcNAc involved modification of their molecular structure. With this aim in view, the glucosamine $1^{14}C$ or the GlcNAc $1^{14}C$ were only added to the mucosal liquid. After 1 h of incubation, the glucosamine and the GlcNAc of the mucosal and serosal fluids were separted from one another on a Dowex column 50 W × 8 H^+ (200–400 mesh)[9]. It was possible to demonstrate that both the glucosamine and the GlcNAc are absorbed without modification of their molecular structure. It was thus excluded that the absorption of GlcNAc involved deacetylation.

In conclusion, the influx of the glucosamine into the intestinal cells is carrier mediated, independent of Na^+ and takes place without active accumulation, GlcNAc is absorbed by a simple process of diffusion without deacetylation of the molecule.

Riassunto. È stato studiato l'assorbimento della glucosamina e della N-acetilglucosamina utilizzando segmenti intestinali tagliati trasversalmente (strips) e sacchetti di intestino tenue di ratto. Il flusso di trasporto della glucosamina nelle cellule intestinali è mediato da carrier, indipendente dallo Na^+ e procede senza accumulo attivo. La N-acetilglucosamina sembra, invece, essere assorbita per mezzo di un semplice processo di diffusione.

L'assorbimento, sia della glucosamina che della N-acetilglucosamina non comporta modificazione della loro struttura molecolare.

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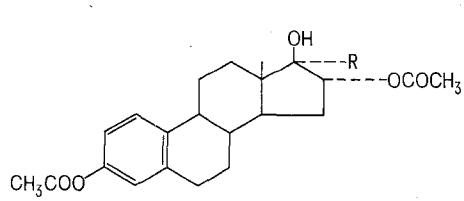
Allene versus Acetylene in Progestational Steroids

In the development of orally active progestational and estrogenic agents, the introduction of the acetylene side chain (17α-ethynyl) was of unique importance. Ethisterone (17α-ethynyl-testosterone) prepared in 1938, was found to be an orally active progestin rather than the expected androgen, and thirty-three years later we find that not only are virtually all contraceptive progestins on the market still of the ethynyl-nortestosterone type[1] but also that the most widely used estrogens (mestranol, ethynyl-estradiol) incorporate this same feature as well[2]. During these years innumerable attempts to find a side chain biologically superior to the original acetylene have resulted in no significant improvement[3]; all the advances being achieved through technologically demanding, expensive 'skeletal' modifications[4].

Some years ago, we made the surprising observation that the allenic compound I obtained as a side product in the preparation of II[5], showed activity in the rabbit deciduoma test[6] (a test in which a progestinestrogen combination or a steroid with a progestational-estrogenic profile would be active) whereas II itself behaved more as an 'impeded-estrogen', typical in fact of these estriol-type compounds.



I R= $-C(CH_3)=C=CH_2$

II R= $-CH_2-C\equiv C-CH_3$

Suspecting that the allene side chain of I might have been responsible for the appearance of a progestational component in the activity and hoping that by attachment of the same allene to a 'progestational' skeleton highly active progestins could be obtained, we prepared[7,8] the 'allenologs' of several known ethynyl-nor-testosterone derivatives and submitted them to the Clauberg assay. Typical responses to the allenologs of norethindrone are shown in Table I.

The remarkable increase in activity found on progressing from acetylene to allenes was also observed in other related tests (e.g., antiestrogenicity[9], inhibition of ovulation[10]). Moreover these increases appear to be quasi-

[1] Several representatives of the other important class (17α-acetoxy-progesterones) have been withdrawn from the market.
[2] For a recent review on acetylenic steroids, see D. Onken and D. Heublein, Pharmazie *25*, 3 (1970).
[3] For an example, see J. H. Fried, T. S. Bry, A. E. Oberster, R. E. Beyler, T. B. Windholz, J. Hannah, L. H. Sarett and S. L. Steelman, J. Am. chem. Soc. *83*, 4663 (1961).
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[5] E. Galantay, USP No. 3, 501, 510 of July 28, 1967.
[6] R. L. Elton, Acta Endocr., Copenh. *51*, 543 (1966).
[7] A highly practical pathway for the preparation of the allenes of the type IV has, as the key step, the $LiAlH_4$ reduction of the quaternary Mannich salts, R = $-C\equiv C-CH_2-\overset{+}{N}R_3$: E. Galantay and D. Habeck, Belgian Patent No. 742,137. The preparation of the type V is exemplified in USP No. 3,541,210.
[8] An alternative pathway to these compounds, described by other workers in USP No. 3,392,165 and 3,392,166, appears to be in divergence to experimental results and its merits or otherwise, can be held in abeyance pending publication of full procedures and results.
[9] R. A. Edgren, D. W. Calhoun, R. L. Elton and F. B. Colton, Endocrinology *65*, 265 (1959).
[10] D. L. Peterson, R. A. Edgren and R. C. Jones, L. Endocr. *29*, 255 (1964).