Methode: Im Laufe der Monate Juli–Dezember 1959 führten wir vier Versuchsreihen mit 250–350 g schweren, weiblichen Meerschweinchen durch. Abgesehen von den Kontrollen erhielten die Tiere während 7 Tagen einmal täglich die zu prüfenden Corticoide, entweder subkutan injiziert oder einzeln verfüttert. Die tägliche Menge war entsprechend den in der Humanmedizin gebräuchlichen Dosen abgestuft: zum Beispiel Hydrocortison $^1/_3$mal schwächer als Cortison, Prednisolon 6mal schwächer, Dexamethason 30mal schwächer usw. (vgl. Tab.). Am 7. Tag erhielten alle Tiere, inkl. die Kontrollen, zusätzlich 1 ml radioaktive, trägerfreie Natriumsulfat-Lösung (^{35}S) intraperitoneal injiziert ($2{,}3 \times 10^5$ cpm). Zwei Tage danach erfolgte die Sektion. Insgesamt kamen 28 Kontrolltiere und 73 Corticoid-Tiere zur Untersuchung. Für jedes Tier wurden die präparierten Kostalknorpel einzeln weiter verarbeitet. Nach Aufschliessen des Knorpelmaterials mit der Methode nach BAILEY[11] und Zugabe von 0,25 ml normaler Natriumsulfat-Lösung als Trägersubstanz wurde der Gesamtschwefel durch Bariumchlorid gefällt. Die Radioaktivität (cpm) des Sulfatniederschlages wurde bestimmt, entsprechend aufgewertet (Halbwertszeit von ^{35}S = 87 Tage) und auf 1 g Knorpeltrockengewicht umgerechnet.

Obwohl in den vier Versuchen gleiche Bedingungen vorlagen, wiesen die Mittelwerte der 6–8 Kontrolltiere jeder Reihe doch gewisse Unterschiede auf. Um nun die cpm-Werte der Corticoid-Gruppen nicht nur innerhalb ihrer eigenen Versuchsreihen mit dem Kontroll-Mittelwert in Vergleich setzen zu können, war es nötig, die für die Kontrolltiere erhaltenen cpm-Werte auf den Mittelwert einer einzelnen Versuchsreihe umzurechnen und danach auch die Werte der einzelnen Corticoid-Gruppen mit dem entsprechenden Rechnungsfaktor zu multiplizieren, bzw. zu dividieren. In der Tabelle ist das so erhaltene Zahlenmaterial niedergelegt.

Ergebnis: Die sechs von uns geprüften Glucocorticoide können in ihrer Wirkung in zwei deutlich unterscheidbare Gruppen eingeteilt werden. Vier der Corticoide ergaben eine statistisch stark gesicherte Hemmung des ^{35}S-Einbaus in den Kostalknorpel, während bei den beiden anderen der ^{35}S-Gehalt des Knorpelmaterials mit jenem der Kontrolltiere übereinstimmte (Tabelle). Es sind dies das natürlicherweise im Organismus vorkommende Hydrocortison und das sich nur durch eine Doppelbindung zwischen C1 und C2 von diesem unterscheidende Prednisolon. Eine Erhöhung der Schwefelaufnahme unter Hydrocortison-Einfluss, wie dies von CLARK und UMBREIT[6] in ihren *in-vitro*-Versuchen beobachtet werden konnte, scheint im lebenden Tier nicht aufzutreten. Die vier hemmenden Glucocorticoide dagegen weisen im Vergleich zu Hydrocortison bestimmte chemische Veränderungen auf: Cortison C11-Ketogruppe, Methyl-prednisolon 6α-CH_3-Gruppe, Triamcinolon 9α-Fluor-16α-OH-Gruppe, Dexamethason 9α-Fluor-16α-CH_3-Gruppe.

RUTH LOTMAR

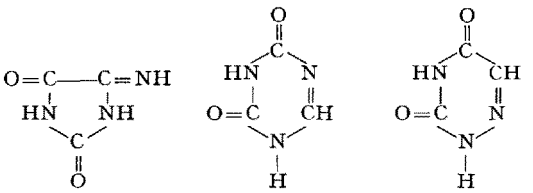
Rheumaklinik und Institut für Physikalische Therapie der Universität Zürich, 24. Februar 1960.

Summary

The uptake of ^{35}S, in the chondroitin sulfate of the costal cartilage of guinea pigs, is inhibited by cortisone, methyl-prednisolon, triamcinolon, and dexamethason. No inhibition could be seen under the influence of the natural adrenal hormone hydrocortisone and its nearest relation, prednisolon.

Antagonism between 5-Azauracil and Pyrimidine Precursors of Ribonucleic Acids in *Escherichia coli*

5-Azauracil was known in the older literature under the name of allantoxaidine[1–3], and was originally considered to be 2,5-dioxo-4-imino-imidazolidine (I). The correct formulation of allantoxaidine structure, however, was arrived at later, and it was BRANDENBERGER and BRANDENBERGER[4], CANELLAKIS and COHEN[5], and most recently GUT *et al.*[6], who showed that allantoxaidine is actually 2,4-dioxo-1,2,3,4-tetrahydro-1,3,5-triazine (II). The results presented in this communication confirm the last-named structure.



I II III

In analogy to 6-azauracil (III) allantoxaidine was later[7] named 5-azauracil.

Biological effects of 5-azauracil were studied by HAKALA, LAW, and WELCH, who found that 5-azauracil in the doses applied does not affect the growth of experimental lymphoma L 1210. On the other hand, ELION *et al.*[8] found that 5-azauracil acts as a powerful inhibitor of growth of adenocarcinoma 755, at non-toxic doses.

As far as the antibacterial effect of 5-azauracil is concerned, no data are available in the existing literature. Therefore, we attempted to study the inhibitory effect of 5-azauracil on the growth of *E. coli* B and devoted our attention to the antagonistic relation of 5-azauracil to a number of purine and pyrimidine components of nucleic acids.

Experimental and Results. The inhibition of growth of *E. coli* B, and the antagonism of 5-azauracil toward purine and pyrimidine derivatives, were studied in a synthetic medium containing glucose and inorganic salts[9]. The bacteria were grown in 10 ml of medium in test-tubes provided with Kapsenberg stoppers at 37°C for 15 h, analogously as in previous work[9]. The growth was estimated turbidimetrically at 575 mμ.

5-Azauracil used in the work described was prepared by a modification[6] of HARTMAN's method[10].

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It was found that a 50% inhibition of growth of *E. coli* B by 5-azauracil is achieved at a concentration of $1{\cdot}25 \times 10^{-4}$ *M*. The relationship of growth inhibition to the concentration of the inhibitor has a different character with 5-azauracil than with 6-azauracil. It was further found that, in the presence of a subbacteriostatic concentration of 5-azauracil, no formation of elongated cellular forms of bacteria takes place, in contrast to what is encountered with 6-azauracil[11]. This finding indicates the existence of a different mechanism of inhibition for 6- and 5-azauracil toward *E. coli*. The *E. coli* cells were found to acquire very rapidly a resistance toward 5-azauracil.

The ability of some pyrimidine derivatives to counteract the growth-inhibition effect of 5-azauracil follows from the data of the Table. Thus it may be seen that 5-azauracil represents for *E. coli* an antimetabolite of ribonucleic acid pyrimidine bases, or rather of their ribosides. Orotic acid, which is known to be a precursor of pyrimidine bases of nucleic acids in numerous biological systems, does not counteract the inhibitory effect of 5-azauracil. This observation can be explained by the inability of *E. coli* cells to utilize exogenous orotic acid for synthesis of pyrimidine bases of nucleic acids, as was shown[12] for the *E. coli* strain 63–86.

The effect of metabolites of nucleic acids on the growth inhibition o *E. coli* B caused by 5-azauracil. 5-Azauracil concentration was 5×10^{-4} M, that of the metabolites also 5×10^{-4} M. The values represent the growth of the culture expressed in % culture density as obtained in the absence of inhibitor

Metabolite added to the medium along with the inhibitor	Growth % control
Adenine	0
Guanine	1
Uracil	114
Cytosine	111
Thymine	2
Orotic acid	0
Adenosine	1
Guanosine	0
Uridine	98
Cytidine	104
Deoxyuridine	85
Deoxycytidine	84
Thymidine	60

The antagonistic effect of deoxyuridine and deoxycytidine can be explained by an utilization of their bases. It is of interest, however, to note the antagonistic effect of thymidine. *E. coli* cells apparently transform thymidine to pyrimidine precursors of ribonucleic acids while thymine cannot be utilized for this purpose.

This work and further experiments will be described in detail in the Collection of Czechoslovak Chemical Communications.

F. Šorm, J. Škoda, and J. Kára

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, December 31, 1959.

Zusammenfassung

Es wurde ein inhibierender Effekt von 5-Azauracil auf Kulturen von *Escherichia coli* B festgestellt, der durch die Anwesenheit von Uracil, Cytosin und ihren Ribosiden aufgehoben wird. Desoxyuridin, Desoxycytidin und Thymidin beseitigen teilweise die hemmende Wirkung von 5-Azauracil.

Adsorption and Active Transport

In a number of ion transport studies it has been found that the kinetic data obtained conform to the Michaelis-Menten treatment of enzyme action[1]. This proved especially valuable in relation to the type of competition exhibited by ions not normally involved in active transport. Thus Epstein and Hagen were able to conclude that in barley roots one type of binding site is specific for K, Rb, and Cs ions since these ions exhibit a competitive type of inhibition, the three as a group differing from Na or Li ions in being bound preferentially. The high degree of specificity found in their study is reflected in the differential uptake of K ions from a medium in which Na ions are preponderant.

Current transport hypotheses localizing the active processes in the cell membrane often seem to imply that the applicability of the Michaelis-Menten treatment indicates the presence of carrier mechanisms. Since the success of this treatment is due solely to the fact that the ion uptake follows a saturation-type kinetics, the same data can be taken as indicating the binding of K or other competing ions onto labile, poison sensitive intracellular adsorption sites. This view is supported below by showing the Michaelis-Menten equation to be formally identical with the Langmuir adsorption formula, thus extending the demonstration of the identity of the latter with the law of mass action[2].

Given a reaction $A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$ where A, B, and C represent molecular species and k_1 and k_{-1} the forward and backward reaction rate constants, one obtains the following expression for the rate of formation of C, the concentrations of A, B, and C being represented by small case letters: $dc/dt = k_1 (a - c) (b - c) - k_{-1} c$.

At equilibrium $dc/dt = 0$ and $(a - c) (b - c)/c = k_{-1}/k_1 = K$. K represents the dissociation constant of the molecular species C, its reciprocal being the equilibrium constant, a measure of the affinity between A and B.

If $a \ll b$, then $(b - c)$ can be taken as approximately equal to b and constant, the formula reducing to $b(a - c)/c = K$, or $c = ab/Kb$, or in the linear form $1/c = 1/b\ K/a + 1/a$.

If in this simple kinetic system one regards A as the enzyme, B its substrate, C denoting the emzyme-substrate complex, the expression represents the Michaelis-Menten treatment applicable to the limiting case when the enzyme-substrate complex does not react with a second substrate to yield the free enzyme and the products, as in the case when the enzyme reacts with an inhibitor.

If on the other hand one regards c as the amount of adsorbed substance in moles per unit volume (i. e. bound to adsorption sites) and a as the total concentration of adsorption sites, also in moles per unit volume, b retaining its original meaning of substrate concentration, then the formula represents a linear transformation of Langmuir's adsorption isotherm.

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