HUMPHREY and TOH[3]. The isolated organ bath, which was similar to that of BOURA, MONGAR, and SCHILD[4], had a volume of 2·5 ml. All manipulations were made by hand. Water was circulated through the warming tubes by a Hoeppler's ultrathermostat. The rats' uteri were brought into oestrus by administering 100 µg of oestradiol during 3 or the same amount of stilboestrol during 2 preceding days. All uteri thus prepared showed the signs of oestrus hyperaemia and very often hydrops. The composition of the Ringer solution for both preparations was that recommended by GADDUM, PEART and VOGT[5] for the isolated rat's colon. For the uterus these authors recommended a similar solution but with 60 mg Ca instead of 30 mg. We obtained better results on uterus using 30 mg Ca. All Ringer solutions contained 22 µg/l of atropine sulphate. The intervals between contractions were 6–8 min for uterus and 5 min for colon. The latter was in contact with solution of 5-HT for 1·5 min. The uterine contractions which reached maximum in less than 30 s, were like those of the colon recorded with the frontal writing lever. The temperature of the bath fluid was 30°C in assays on uterus and 25°C on colon. Two doses of the standard and two of the unknown were used in each assay. The ratio of high to low dose was always 2:1 except in one assay where it was 1·5:1. Each assay consisted of 4 to 8 groups of contractions. In a few experiments the first 4 contractions were omitted when calculating the result. The limits of error and activity ratio were estimated by analysis of variance and the methods of calculation given by SCHILD[6]. The concentrations of 5-HT refer to the pure base.

To show which preparation is preferable for quantitative assay of 5-HT we applied 2 + 2 procedure both to rat's uterus and to colon. We assayed the concentration of 5-HT solution of known strength. 19 such assays on colon and 13 on uterus were made. The assays are unselected. The arithmetic means of the deviations from the true result are – 2·184% for colon and + 2·453% for uterus. The corresponding average limits of error (P = 0·05) are 8·8% and 10·5%. The harmonic mean of the index of precision $(\frac{s}{b})$ is 0·035 for colon and 0·047 for uterus.

The results show that rat's uterus is as reliable for a quantitative assay as the colon, the actual deviations from the true result being not significantly different. The use of the uterus is, however, more laborious because one in 3 uteri must be discarded owing to irregular responses, higher doses sometimes giving a smaller effect than lower, whereas almost every colon is suitable for the assay. We were, moreover, obliged to use higher concentrations for the uterus: the average concentration used for the uterus was 87 µg/ml, whereas for the colon it was 26 µg/ml. Owing to these advantages the colon is to be preferred for 2 + 2 assay of 5-HT.

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Department of Pharmacology, Medical Faculty, University of Zagreb, Yugoslavia, November 7, 1955.

Zusammenfassung

Die zwei meistgebrauchten Methoden der biologischen Auswertung von 5-Hydroxytryptamin, nämlich die am isolierten, atropinisierten Rattenuterus bzw. Rattencolon, wurden in bezug auf ihre Eignung für die quantitative Bestimmung verglichen. Für diesen Zweck bedienten wir uns der sogenannten 2 + 2-Dosen-Prozedur. Beide Methoden ergaben statistisch gleichwertige Resultate. Die Unregelmässigkeit der Uteruskontraktionen machen das Testieren öfters unmöglich, was beim Colon sehr selten der Fall ist. Ausserdem ist das Colon empfindlicher als der Uterus. Wegen dieser Vorteile ist das Colon für die quantitative biologische Auswertung von 5-Hydroxytryptamin dem Uterus vorzuziehen.

[3] J. H. HUMPHREY and C. C. TOH, J. Physiol. *124*, 300 (1954).

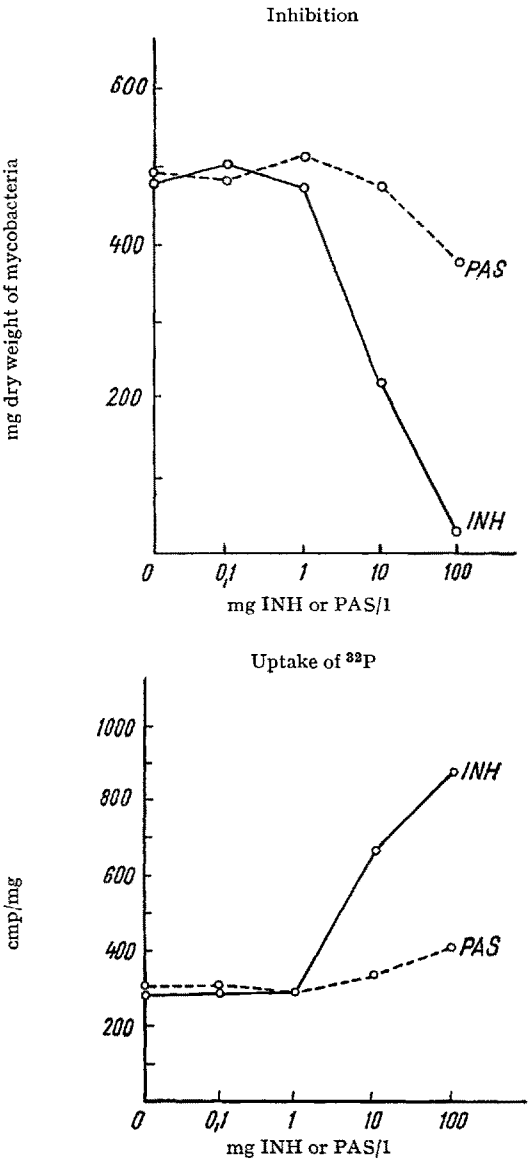
[4] A. BOURA, J. L. MONGAR, and H. O. SCHILD, Brit. J. Pharmacol. *9*, 24 (1954).

[5] J. H. GADDUM, W. S. PEART, and M. VOGT, J. Physiol. *108*, 467 (1949).

[6] H. O. SCHILD, J. Physiol. *101*, 115 (1942).

On the Mode of Action of Synthetic Antituberculotica

When studying the mode of action of synthetic tuberculostatica, i.e. INH and PAS, we came to the conclusion in our experiments that the use of concentra-



Saprofytic mycobacterium S incubated 7 days by 37°, grown on Long's medium (50 ml) with 0·25 µc ^{32}P/ml. cpm/mg = counts per minute per mg dry weight of mycobacteria.

tions of these drugs resulting in partial growth inhibition affected the uptake of phosphorus by the mycobacterial cell. We worked with a saprophytic strain "S" as the sensitive test-organism of our experiments, using the specific effect described earlier of these compounds on the genus mycobacterium[1]. Using the radioisotope ^{32}P as the indicator of phosphorus-quantity bound by the cells, we found an increased uptake of ^{32}P after the application of partial growth-inhibiting concentrations of INH and PAS, or else its accumulation in the mycobacterial cell. The following graphs show the relation between the growth-inhibition induced with INH or PAS and the uptake of ^{32}P in the mycobacterium "S".

From the graphs it is obvious that the increased ^{32}P uptake is strongly dependent on the rate of growth-inhibition. Because a similar increase of ^{32}P uptake or accumulation was also registered when using penicillin (300 i.u.) for 1 h on a strain of *Staphylococcus aureus* (a 24-h-old culture growth on broth in our experiments), a wellknown fact explainable by an enormous increase of nucleic acid as the result of penicillin action[2], we suppose that this fact, when induced with compounds of different structural composition, may be considered more generally than was the case until now. On the other hand, some Japanese authors observed that oxytetracycline exerts an inhibiting action on the incorporation of ^{32}P by *Staphylococcus aureus* strains[3].

There are some theoretical possibilities to explain the above-mentioned observed fact. One of them, based firstly on its unspecificity and secondly on some new investigations dealing with the problem of the high-energy phosphorus bounds[4] may be described as follows:

(1) The antituberculotica used do not destroy the synthesis of the high-energy phosphorus compounds (the oxidative phosphorylation);

(2) nevertheless the resolution of the energy of these compounds is decreased and thus accumulation of them occurs in the cells[5].

Further experiments are needed to show whether this hypothesis is right.

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Zusammenfassung

Beim Studium des Wirkungsmechanismus der synthetischen Tuberkulostatika INH und PAS wurde festgestellt, dass die inhibierenden Konzentrationen dieser Substanzen eine Steigerung des Verbrauches (evtl. Anhäufung) des Radiophosphors ^{32}P in Zellen der Mykobakterien verursachen. Diese Steigerung des ^{32}P-Verbrauches steht proportionell zur Inhibitionsstufe.

[1] A. LEMBKE, E. KRÜGER-THIEMER, R. B. KUHN, and W. UECKER, Schweiz. Z. allg. Path. Bakt. *16*, 222 (1953).

[2] L. ETTLINGER, Schweiz. med. Wschr. *85*, 271 (1955).

[3] Y. MIURA, Y. NAKAMURA, Y. YOSHIZAWA, and H. MATSUDAIRA, Antibiot. and Chemother. *3*, 822 (1953).

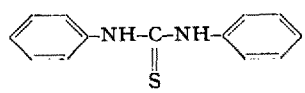
[4] H. A. KREBS. Brit. med. Bull. *9*, 97 (1953). – A. KLEINZELLER, Chemické listy *49*, 376 (1955).

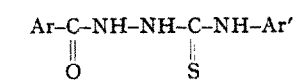
[5] M. MACHEBŒUF, Bull. Soc. chim. France *30*, 161 (1948).

Activité *in vivo* de dérivés de la thiourée contre le virus de l'influenza[1]

Les recherches sur les composés chimiques possédant une activité antivirale, en particulier en ce qui concerne le virus de l'influenza, ont surtout porté jusqu'à présent sur les antimétabolites, tels que les antagonistes d'acides aminés ou de bases puriques indispensables[2]. D'autre part, les tests généralement utilisés, et qui étaient basés sur l'inhibition du développement du virus en cultures de tissus ou sur embryon de poulet, ne donnaient que peu d'indications sur la portée pratique de cette inhibition, et un grand nombre de substances ainsi reconnues comme actives se sont révélées inefficaces contre l'infection chez l'animal[3].

Récemment, en recourant directement à un test chimiothérapeutique chez la souris, nous avons pu déceler une activité contre le virus de l'influenza chez plusieurs dérivés de la thiourée, en particulier chez le 4-chloro-4'-fluorothiocarbanilide[4]. Depuis, nous avons étendu cette étude à de nombreux composés analogues, afin de rechercher des relations entre constitution chimique et activité antivirale dans ce domaine. Les nouvelles substances ainsi examinées sont (à trois exceptions près) des molécules renfermant un ou deux groupements NH-CS-NH-. Ce sont principalement: 1° des dérivés substitués du thiocarbanilide (I); 2° des 1-acyl-4-arylthiosemicarbazides de formule générale (II); 3° des 1-(arylaminothioformyl)-thiosemicarbazides (III); 4° des 4-aryl-1-(arylaminothioformyl)-thiosemicarbazides (IV); et 5° des *bis*-anilides (V) de l'acide thiocarbohydrazide-*bis*-1, 5-thiocarbonique.


(I)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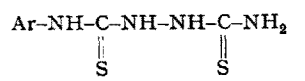
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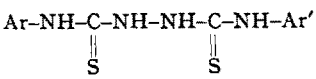
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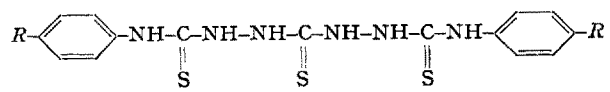
(V)

Les composés (I), (II), (III), (IV) et (V) ont été préparés en faisant réagir des isothiocyanates d'aryles respectivement sur des arylamines[5], des hydrazides[6], la thio-

[1] Communication présentée au Congrès international de Chimie pure et appliquée de Zürich (Juillet 1955).

[2] *Cf.* I. TAMM, K. FOLKERS et F. L. HORSFALL jr., Yale J. Biol. Med. *24*, 559 (1952); J. exper. Med. *98*, 219 (1953). – I. TAMM, K. FOLKERS, F. L. HORSFALL jr. et C. H. SHUNK, J. exper. Med. *99*, 227 (1954). – C. HANNOUN, C. r. Acad. Sci. *236*, 864 (1953).

[3] *Cf.* K. K. TAKEMOTO, M. L. ROBBINS et P. K. SMITH, J. Immunol. *72*, 141 (1954).

[4] N. P. BUU-HOÏ, P. GLEY, N. D. XUONG et A. BOUFFANAIS, C. r. Acad. Sci. *238*, 2582 (1954).

[5] *Cf.* N. P. BUU-HOÏ, N. D. XUONG et N. H. NAM, J. chem. Soc. *1955*, 1573.

[6] N. P. BUU-HOÏ, N. D. XUONG et N. H. NAM, C. r. Acad. Sci. *238*, 295 (1954).