The mechanism by which PABA increased the activity of isonizid is still unknown. It was, however, suggested by KUBALA[4] that this might have been explained by competitive acetylation of PABA instead of isoniazid, which was known to operate *in vivo*[5] and undoubtedly could have occurred also in the conditions *in vitro*.

The reliability of this hypothese was first checked in an experiment where the synergistic influence of both PABA and 4-acetamido-benzoic acid (Ac-PABA) on the isoniazid activity was compared.

The strains *Mycobacterium 607* and H 37 Rv were maintained by serial passages on the surface of the Proskauer-Beck synthetic liquid medium without albumine or serum. This medium (pH 6.8) was distributed in 50.0 ml amounts in 250 ml Erlenmeyer flasks, to which isoniazid alone or with either PABA or Ac-PABA was added, and seeded with one loop of a 7-days old culture of the *M. 607* strain or a 14-days old culture of the H 37 Rv strain. Isoniazid was sinter-sterilized and added aseptically to the medium, PABA and Ac-PABA were added to the medium before autoclaving. All experiments were run in four parallel flasks. After 7 days incubation at 37°C for the *M. 607* strain or 14 days incubation for the H 37 Rv strain all cultures were autoclaved and the bacterial mass was separated by filtration from the medium. Further description of the method may be found elsewhere[1]. Concentrations of the drugs as well as results of the experiments are summarized in Table I and II.

Table I

Drug	Concentration in μg/ml	Dry weight of bacteria in g[a, b]
Control	—	0.5424
PABA	200	0.5290
Ac-PABA	260[c]	0.5513
Isoniazid	1	0.0692
Isoniazid + PABA	1 + 200	0.0316
Isoniazid + Ac-PABA	1 + 260	0.0888

[a] Strain *Mycobacterium 607*.
[b] Mean value from four parallel flasks.
[c] Equimolar amount to 200 μg of PABA.

Table II

Drug	Concentration in μg/ml	Dry weight of bacteria in g[a, b]
Control	—	0.2933
PABA	200	0.2849
Ac-PABA	260[c]	0.2906
Isoniazid	0.05	0.0581
Isoniazid + PABA	0.05 + 200	0.0333
Isoniazid + Ac-PABA	0.05 + 260	0.0599

[a] Strain H37Rv.
[b] Mean value from four parallel flasks.
[c] Equimolar amount to 200 μg of PABA.

It may be seen from both Tables that, in both the *M. 607* or the H 37 Rv strain, Ac-PABA was ineffective in increasing the tuberculostatic properties of isoniazid, whereas PABA showed the previously described synergistic influence on the isonizid activity. These results appear to offer some evidence in favour of the afore mentioned competitive acetylation hypothesis. Nevertheless, a direct proof of acetylation of either isoniazid or PABA by mycobacterial cultures *in vitro* was definitely needed. It is difficult to trace acetylated isoniazid under the conditions of our experiments. We attempted therefore to prove the formation of Ac-PABA from PABA in mycobacterial cultures *in vitro*.

The Proskauer-Beck medium (pH 6.8), to which 200.0 μg of PABA per ml were added before autoclaving was distributed in Erlenmeyer flasks and seeded as described above. After incubation for the afore-mentioned time intervals, the cultures of both strains were autoclaved and the bacterial mass was separated by filtration from the medium. The filtrates, each time from a control medium and from the medium with PABA, were further investigated as to their content of Ac-PABA by paper chromatography. The chromatograms were run ascendently on No. 3 Whatman paper with use of *n*-propanol, water, and ammonia solvent (50 : 38 : 12). Ac-PABA was hydrolysed on the paper by spraying with 10% hydrochloric acid and exposing it for 10 min over boiling water. The spots were detected by dipping the paper into the Ehrlich reagent with acetone[6].

Our attempts to demonstrate Ac-PABA in culture filtrates of both strains remained, however, unsuccessfull. Thus we may conclude that under the conditions of our experiment PABA is not acetylated by either strain *in vitro*, and the competitive acetylation hypothesis of increasing the bacteriotropic effect of isoniazid in the presence of PABA can hardly be maintained.

Our experiments, however, have shown that Ac-PABA does not increase the effect of isoniazid on either of the strains used. Thus we may suggest that *in vivo*, where acetylation of PABA undoubtedly takes place, the combination of isoniazid and PABA will probably be no more effective than isoniazid alone, except perhaps with very high doses of PABA. This suggestion has been verified[7].

Zusammenfassung. 4-Acetaminobenzoesäure hat *in vitro* im Unterschied zu 4-Aminobenzoesäure, keinen günstigen Einfluss auf die antituberkulotische Wirkung von Isoniazid. Bei den Stämmen *Mycobacterium 607* und H 37 Rv wurde jedoch chromatographisch keine Bildung acetylierter 4-Aminobenzoesäure nachgewiesen.

R. URBANČÍK, P. KRAUS, and Z. ŠIMÁNĚ

Tuberculosis Research Institute, Prague-Bulovka (ČSR), September 28, 1960.

[4] E. KUBALA, Amer. Rev. Tuberc. *78*, 949 (1958).
[5] J. C. BELL, D. K. RIEMENSNIDER, and R. S. MITCHELL, Amer. Rev. Tuberc. *76*, 152 (1957).
[6] J. B. JEPSON, Lancet *ii*, 1009 (1955).
[7] R. URBANČÍK, Z. ŠIMÁNĚ, P. KRAUS, and I. REIL, Exper. *17*, 84 (1961).

Isoniazid and 4-Aminobenzoic Acid in the Treatment of Murine Tuberculosis[1]

As shown previously, 4-aminobenzoic acid (PABA) increases the tuberculostatic activity of isoniazid *in vitro*[2]. It was now of interest to investigate the effect of isoniazid combined with PABA in the conditions *in vivo*.

[1] Part of these experiments was performed in the Institute of Microbiology, University of Brno.
[2] R. URBANČÍK, Amer. Rev. Tuberc. *76*, 706 (1957).

No. of mice	Group	Dosage	ST_{50} [a]	99% confidence interval [b]
12	Control	—[c]	19.4	
10	PABA	0.5 mg	16.5	
13	INH	100 μg	36.0	$33.04 < \mu < 38.96$
11	INH + PABA	100 μg + 0.5 mg	38.2	$35.58 < \mu < 40.78$
13	Control	—	13.1	
11	PABA	1.0 mg	10.3	
12	INH	100 μg	30.6	$22.11 < \mu < 39.21$
12	INH + PABA	100 μg + 1.0 mg	29.6	$21.28 < \mu < 37.88$
13	Control	—	15.6	
10	PABA	3.0 mg	8.9	
15	INH	100 μg	22.2	$18.15 < \mu < 26.11$
14	INH + PABA	100 μg + 3.0 mg	30.9	$28.26 < \mu < 33.58$

[a] Median survival time expressed in days.
[b] Calculated with use of the *t*-test and expressed in days.
[c] To all mice in all control groups plain saline was administered daily substaneously for 14 days.

White mice of the 'H' strain, weighing 16 to 20 g were infected intravenously with 0.5 ml of a 10-days old Dubos culture of the H37Rv strain of *M. tuberculosis*. The animals were always distributed by random selection into groups of at least 10 mice and were fed in the course of the treatment with rolls and water *ad libitum*. The therapy was started the day after the infection and lasted 14 days. Isoniazid and PABA[3] were administered subcutaneously, each of them separately in one daily dose. Three sets of experiments were performed at different time intervals, each of them with a different dose of PABA. The median survival time was registered; the results, which were analysed with use of the *t*-test[4], are summarized in the Table.

It may be seen that with 0.5 mg or 1.0 mg of PABA no increase could be observed in the therapeutic activity of isoniazid, if compared with the therapeutic effect of isoniazid alone. There is, however, a statistically significant difference in the therapeutic activity of isoniazid combined with 3.0 mg of PABA, if compared with isoniazid alone.

The mechanism of this phenomenon is not clear. It has been suggested that this increase in activity of isoniazid in the presence of PABA might be attributed to the competitive acetylation of PABA instead of isoniazid, which is known to operate *in vivo*[5], but could not be proved to take place *in vitro*[6]. There are, however, some suggestions that PABA cannot be acetylated by tuberculous animals[7]. Thus this question still remains open.

As discussed in a previous communication[8], PABA decreases the median survival time of tuberculous mice. This phenomenon, for which we still have no explanation, may readily be observed in the Table presented.

Zusammenfassung. Bei der Mäusetuberkulose war die kombinierte Therapie von Isoniazid mit 4-Aminobenzoesäure (PABA) (3 mg/Tag/Tier) wirksamer als diejenige von Isoniazid allein. Bei niedrigerer PABA-Dosis wurde der Synergismus nicht nachgewiesen.

PABA allein verkürzt die mittlere Überlebenszeit bei tuberkulösen Mäusen.

R. Urbančík, Z. Šimáně, P. Kraus, and I. Reil

Tuberculosis Research Institute, Prague-Bulovka (ČSR), September 29, 1960.

[3] Sodium salt of PABA, produced as 'Paraminan'® by R. Gallier, Paris.
[4] E. Weber, *Grundriss der biologischen Statistik* (G. Fischer, Jena 1956), p. 158.
[5] J. C. Bell, D. K. Riemensnider, and R. S. Mitchell, Amer. Rev. Tuberc. *76*, 152 (1957).
[6] R. Urbančík, P. Kraus, and Z. Šimáně, Exper. *17*, 83 (1961).
[7] S. P. Martin, S. N. Chaudhuri, C. D. Cooper, and R. Green, Ciba Foundation Symposium on Experimental Tuberculosis (1955), p. 102.
[8] R. Urbančík, Jap. J. Tuberc. *6*, 95 (1958).

Über die Bindung von Kallidin an Heparin

Nach früheren Beobachtungen [1,2] bildet Heparin mit Histamin eine nichtdialysierbare Verbindung, in der die beiden Stoffe mit grösster Wahrscheinlichkeit auch in den Mastzellen gespeichert sind[3]. Wir haben nun festgestellt, dass auch das Polypeptid Kallidin, das chemisch und pharmakologisch von Bradykinin nicht unterscheidbar ist[4], mit Heparin[5] einen undialysierbaren Komplex bildet und wie Histamin durch die Verbindung 48/80 wieder freigesetzt werden kann. Der Kallidin-Heparin-Komplex ist auch aus sehr verdünnter Lösung durch Alkohol fällbar.

Bei der stehenden Dialyse wässeriger Kallidinlösungen durch Cellophanmembranen (Volumenverhältnis Innen- zu Aussendialysat zum Beispiel 1:4) findet man unter unseren Versuchsbedingungen[6] auch in Abwesenheit von Heparin nach 24 h nicht wie erwartet 80% Kallidin aussen und 20% innen, sondern nur etwa 10% aussen und 0% innen. Das Kallidin ist nicht zerstört, sondern an die Cellophanmembran adsorbiert und kann durch Elution der zerschnittenen Membran mit *n*-NH_3 wiedergewonnen werden.

In Gegenwart von Heparin (bis zur unteren Grenze von 2,5 IE Heparin pro 1 Einheit Kallidin) findet sich die gesamte eingesetzte Kallidinmenge im Innendialysat. Dies besagt, dass die Dissoziationskonstante der Kallidin-Heparin-Verbindung äusserst niedrig sein muss, weil andernfalls nach einigen Stunden das Kallidin trotz der Gegenwart von Heparin an die Cellophanmembran adsorbiert sein müsste. Da die biologische Aktivität des Kallidins in seiner Bindung an Heparin nicht beeinträchtigt ist, erfolgt die Anlagerung des Kallidins so, dass zwar die Diffusion, nicht aber die Anlagerung an den Rezeptor der Uterus- oder Darmwand behindert ist. Das durch Heparin gebundene Kallidin ist nur in geringem Masse gegen den Angriff der Kallidinase geschützt. Bei Anwesenheit von Heparin und Verbindung 48/80 (Mengenverhältnis zum Beispiel 30 γ Verbindung 48/80 auf 20 IE Heparin) verhält sich Kallidin im Dialyseversuch wie für freies Kallidin geschildert.

Versuchsbeispiel zur Fällung von Kallidin durch Heparin: 100 Einheiten Kallidin + 1000 Einheiten Heparin gelöst in je 1 ml Wasser + 8 ml 96%iger Alkohol. Der sofort

[1] R. Amann und E. Werle, Klin. Wschr. *34*, 207 (1956).
[2] E. Werle und R. Amann, Klin. Wschr. *34*, 624 (1956).
[3] A. Schauer und E. Werle, Z. ges. exp. Med. *131*, 100 (1959).
[4] M. Schachter, in *Polypeptides which Affect Smooth Muscles and Blood Vessels* (Pergamon Press, London 1960), p. 199.
[5] Verwendet wurden Heparin-Novo (5000 IE/ml) und Heparin Hoffmann-La Roche in Substanz (113 IE/mg).
[6] (10–20 Kallidin-Einheiten in ca. 5 ml). 1 Einheit Kallidin ist diejenige Kallidinmenge, die man aus 1 ml Rinderplasma mit 1 Einheit Kallikrein aus Schweine-Submaxillarisdrüsen bei 20°C freisetzen kann.
[7] R. Jaques, K. Küttner, H. J. Bein und R. Meier, Exper. *16*, 147 (1960).
[8] R. Amann und E. Werle, Klin. Wschr. *35*, 22 (1957).