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.

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[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).

sich bildende weisse Niederschlag wird abzentrifugiert und in wenig Wasser aufgenommen. Die Lösung enthält rund 90% der eingesetzten Kallidinmenge. Diese empfindliche Fällungsreaktion dürfte auch für die Isolierung von Kallidin von praktischer Bedeutung sein.

JAQUES et al.[7] haben kürzlich festgestellt, dass auch Hypertensin durch Heparin gebunden wird. Da ausser Histamin, Hypertensin und Kallidin anscheinend auch Thrombin[8] mit Heparin einen Komplex bildet, nehmen wir mit JAQUES et al. an, dass der Bildung von Heparin-Komplexen eine allgemeinere Bedeutung zukommt.

Summary. Kallidin gives, with heparin, a non-dialysable complex, which can be precipitated with alcohol. In this binding, kallidin is pharmacologically active, but may be split off by the substance 48/80.

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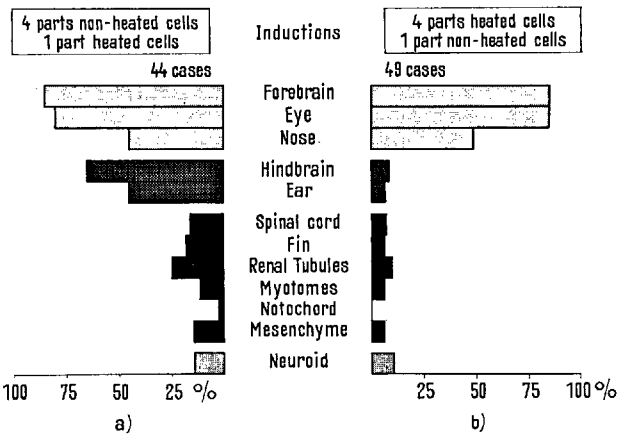
Quantitative Evidence for the Two-Gradient Hypothesis in the Primary Induction

The idea of a two-gradient system acting in the primary induction, presented by LEHMANN[1] and YAMADA[2], had earlier led us to experiments in which two heterogeneous tissues of qualitatively different inductive action were implanted simultaneously in order to test their combined effect[3]. A new type of induction was observed, explained as being a combined action of two principles, one a mesodermalizing and the other a neuralizing inductive system. A very similar theory was simultaneously presented by YAMADA and TAKATA[4].

These ideas were based on two types of experiment: The combination of two different inductors, or a separation of the two different inductive effects by chemical treatment of one inductor tissue, originally 'combined'. None of these experiments, however, could give us quantitative information on the hypothetical principles operating in the primary induction. In such a quantitative operation, our present methods with their marked limitations seem to offer only two possible ways of approach; either the size of the implant is varied, or different concentrations of the active factors incorporated in some non-inducing material are used. For a number of reasons, both of these methods have to be considered inadequate, and also the techniques give rise to a variety of technical errors. Another set-up was accordingly planned so as to get at least some relative data on the amount of the different inducing principles, and the significance of their ratio to the induction process.

Method. HeLa-cells cultured in a medium containing human serum were used as inductors[5]. After mechanical removal of the cells from the walls of the culture flasks, they were treated as follows:

From 10 to 11 million cells were collected from 3 culture flasks, washed several times with saline solution, and finally dispersed in 10 cm³ of saline. Of this homogeneous cell suspension, 5 cm³ was heated on a waterbath for 30 min at 65°—a treatment which is known to inactivate the mesodermalizing capacity of cells and tissues[5,6]. Meanwhile, the other half of the suspension was kept in an incubator at 37°C. Following this, the two suspensions were combined in two test tubes in the following ratios: A) 1 cm³ heated cells + 4 cm³ non-heated cells; B) 4 cm³ heated cells + 1 cm³ non-heated cells.



Percentage of the induced structures in the experimental series.

During the whole of this procedure, every care was taken to keep the cell suspensions dispersed homogeneously, and the tubes were repeatedly shaken between the different stages of treatment. The two combined suspensions (A, B) were then simultaneously centrifuged at 5000 rpm, and the compact sediment covered with cold 70% alcohol. After 4 to 6 h of alcohol treatment, the cells were washed and used in explantation experiments according to 'sandwich' method[7]. The presumptive epidermis of young Triturus vulgaris-gastrulae was used, and the explants were cultivated for 10 days in Holtfreter-solution. The differentiations were analyzed microscopically from serial sections.

Results. The results are demonstrated in the Figure. Only one comment has to be made as regards the Figure. As was the case in our earlier works, only the presence or absence of the different induced structures was recorded, and thus the analysis gives no information on the amount or size of the secondary formations. For instance, the large masses of striated muscle seen in some cases in series A were recorded as being equal to the explants in series B, where often only a few muscle fibers were found. Thus, in this respect the title of our report is misleading, and the analysis of the inductions is by no means quantitative.

Discussion. In comparison with our earlier findings obtained with HeLa-cells in implantation experiments[5], the low frequency of mesodermal structures was conspicuous in both of the present series. This is partly correlated with the generally known fact that mesodermalization is always weaker in explantation experiments than in those of implantation[3,8]. Furthermore, we know from our earlier and partly unpublished experiments that a maximal mesodermalizing effect is noted in HeLa-cells, only if their culture medium is changed shortly before the experiment.

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