lymphosarcome de Murphy-Sturm, cette tumeur finissait toujours par disparaître après traitement intense au polysaccharide, mais cela se produisait de façon différente suivant l'hormone injectée. Il faut dire que la tumeur dont nous nous servions régressait souvent sous l'effet des médications adjuvantes seules.

Des expériences ont été effectuées chez des rates portant la tumeur de Walker 256. Dans cette série le choc initial pouvait être moins marqué, mais les rates survivaient assez facilement, à moins que les tumeurs soient beaucoup plus grosses ou qu'on ait ajouté des médications qui accentuaient le stress. Dans aucun cas d'ailleurs, cette dernière tumeur n'a régressé complètement, même si certaines médications causaient une involution initiale prononcée. On sait déjà l'action préférentielle des polysaccharides au niveau des sarcomes.

Les polysaccharides que nous utilisions avaient été isolés deux ans auparavant et gardés ensuite à la température du laboratoire, et comme on a déjà remarqué[8] que le pouvoir actif anti-tumoral diminuait avec le temps dans ces préparations, cela peut expliquer pourquoi nous devions utiliser des doses beaucoup plus fortes et moins critiques que celles qu'on emploie généralement. Nous avons remarqué d'ailleurs que nos préparations une fois dissoutes (nous dissolvions la poudre de polysaccharide dans une quantité minime d'eau distillée), perdaient rapidement après quelques jours tout pouvoir de provoquer un choc chez l'animal cancéreux.

Dans les mêmes circonstances expérimentales, nous avons aussi utilisé à hautes doses une préparation de polysaccharide, également vieille de deux ans, extraite de culture de *Serratia marcescens*; celle-ci ne provoquait pas de choc initial et la mort des animaux, si elle survenait, n'apparaissait que tardivement au bout de 24 à 72 h, due probablement aux métabolites provenant de la tumeur nécrosée.

Summary. Aged preparations of polysaccharide from *E. coli* were highly effective in inducing a lethal shock in rats bearing a tumor. This effect may be increased by some medications, while various steroid hormones afforded an efficient protection.

P. LINDSAY

Faculté de Médecine, Université Laval, Québec (Canada), le 21 avril 1961.

[8] H. J. CREECH, L. H. KOEHLER, H. F. HAVAS, R. M. PECK et J. ANDRE, Cancer Res. *14*, 817 (1954).

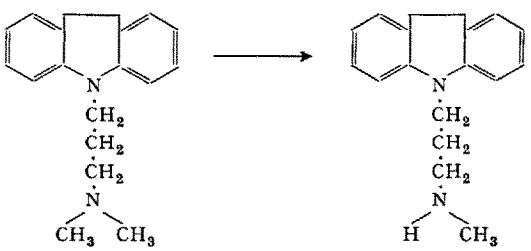
Isolation from Rat Brain of a Metabolic Product, Desmethylimipramine, that Mediates the Antidepressant Activity of Imipramine (Tofranil)[1]

The effectiveness of imipramine (Tofranil) in the treatment of endogenous depression, first reported by KUHN[2], has now been confirmed by numerous clinical studies. The drug exerts weak chlorpromazine-like effects in normal animals and man, while it exerts an antidepressant action in depressed patients only after repeated doses over a period of several weeks. Recent studies from this laboratory have shown that imipramine counteracts the syndrome of central effects elicited in rats by reserpine and by a synthetic benzoquinolizine, without inhibiting the actions of chlorpromazine[3]. Again, there is a delay in onset of action, several hours elapsing before this action appears. These considerations suggested to us that both the anti-reserpine and the clinical antidepressant effects of imipramine might be mediated through a metabolic product.

The present communication describes the isolation and identification from brain of a metabolite of imipramine which appears to be responsible for the antidepressant action of the drug. The metabolite, desmethylimipramine (DMI), is derived from imipramine *in vivo* by the removal of one methyl group from the sidechain nitrogen as follows:

The antidepressant activity of imipramine was determined in male Sprague Dawley rats (160–180 g) by the prevention or reversal of the reserpine-like syndrome[4] elicited by the intraperitoneal administration of 10 mg/kg of RO-4-1284[5], or the intravenous injection of 2 mg/kg of reserpine. The concentration of imipramine in brain was assayed by a fluorometric procedure[6].

Brain levels of imipramine were measured in rats after the intraperitoneal injection of 40 mg of imipramine per kg. The drug readily entered the brain and in 45 min had attained a level of 14 μg/g; the level then declined rapidly and in 3 h was less than 3 μg/g. If RO-4-1284 was injected 45 min after imipramine, the latter compound had no obvious effect on the reserpine-like syndrome elicited by the benzoquinolizine. However, if RO-4-1284 was given 3 h after imipramine, the central effects of the benzoquinolizine were now prevented even though the imipramine level was less than 3 μg/g of brain. From these results it is clear that the anti-reserpine activity of imipramine was not directly related to its concentration in brain.



Imipramine Desmethylimipramine (DMI)

[1] Presented in part as an abstract in Fed. Proc. *20*, 321 (1961). Some of the material in this communication is taken from a dissertation by J. V. DINGELL to be submitted to the Graduate School of Georgetown University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] R. KUHN, Schweiz. med. Wschr. *87*, 1135 (1957).

[3] F. SULSER, J. WATTS, and B. B. BRODIE, N. Y. Acad. Sci., in press.

[4] Rats treated with RO-4-1284 (10 mg/kg i.p.) or reserpine (2 mg/kg i.v.) exhibit blepharospasm and an almost complete lack of spontaneous motor activity, remaining motionless in a peculiar hunched-back position. In addition they show little response to stimuli such as sound or touch. In imipramine pretreated animals, these signs are prevented or reversed. Reversal is evidenced by a pronounced and persistent hyperactivity of a 'compulsory' exploratory nature.

[5] The reserpine-like properties of synthetic benzoquinolizines were first described by A. PLETSCHER et al., Arch. exp. Path. Pharmakol. *232*, 499 (1958). The chemical structure of RO-4-1284 is 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydrobenzo(a)-quinolizine. This compound was generously donated by Hoffmann-La Roche Inc.

[6] J. R. GILLETTE, J. V. DINGELL, and G. P. QUINN, Fed. Proc. *19*, 137 (1960).

The presence of considerable amounts of a metabolic product in brain was established as follows: Pooled brains from five animals given imipramine 3 h previously were homogenized with three volumes of water, the homogenate was alkalinized with 1.0 *N* NaOH to about pH 12, and shaken with three volumes of heptane containing 1.5% isoamyl alcohol. Phenolic material was removed by shaking the heptane phase with a small volume of 0.1 *N* NaOH. Basic material in the heptane phase was returned to an aqueous phase by shaking with $^1/_3$ volume of 0.2 *M* phosphate buffer, pH 5.9, which would extract only a small fraction of any imipramine that might be present. Traces of imipramine in the buffer phase were removed by shaking with three volumes of the heptane-isoamyl alcohol mixture, and the buffer phase was then alkalinized with 3 *N* NaOH. The fluorescence spectra of this solution, examined with a spectrofluorometer, revealed material with fluorescent characteristics similar to those of imipramine (activation maximum: 295 mμ, fluorescence maximum: 415 mμ, uncorrected). The results indicated that a heptane-soluble metabolite had accumulated in brain in amounts equivalent to about 10 μg of imipramine per g of tissue. The material was then extracted into heptane.

The metabolite was identified as desmethylimipramine (DMI) as follows:

Gas Chromatography. An aliquot of the heptane phase was evaporated to dryness under reduced pressure, and the residue dissolved in a small amount of acetone. The resulting solution was dried over $MgSO_4$ and evaporated to about 0.1 ml at room temperature under a stream of nitrogen. The material was subjected to gas chromatography at 175°C using a column of 0.75% Silicone Polymer, SE 30 Gas-Chrome P (mesh-size 100–140), and an argon pressure of one atmosphere[7]. The retention time of the principal component was identical to that of DMI, the monomethyl analogue of imipramine, a metabolite previously isolated by HERRMANN and PULVER[8] from urine, lung, and liver of animals given imipramine.

Paper Chromatography. Another aliquot of the heptane phase was evaporated to dryness under reduced pressure and the residue dissolved in a small volume of methanol. The methanol solution was applied to Whatman 3 paper, impregnated with peanut oil[9]. The chromatograms were developed by ascending chromatography with a solvent system consisting of concentrated ammonia and methanol (1:1). The chromatogram was irradiated with a shortwave ultraviolet lamp (Model SL 2537 Mineralight lamp). No fluorescent material was seen at room temperature, but on cooling the paper with liquid nitrogen, a brilliant fluorescent spot appeared with an Rf value (0.23) corresponding to DMI. No fluorescent spot was found having an Rf value (0.33) corresponding to desdimethylimipramine, the primary amine analogue of imipramine. When the chromatogram was sprayed with diazotized *p*-nitroaniline and HCl as described by HERRMANN et al.[9], the fluorescent material formed a blue spot similar to that formed from authentic DMI.

The accumulation of DMI in brain after administration of imipramine suggested that the action of the latter drug might be mediated through the metabolic product. Further experimental support of this was provided by giving imipramine in repeated doses, 20 mg/kg, every 6 h over a period of 24 h. 4 h after the last dose, the animals were given either RO-4-1284 or reserpine. No sedation occurred, and in about 1 h the animals displayed marked hyperactivity lasting over several hours. Analysis of brain showed negligible amounts of imipramine and about 20 to 30 μg of DMI per g of tissue.

Pharmacological studies have shown that DMI is a new type of antidepressant[10]. It does not elicit excitation in the normal rat nor does it block monoamine oxidase or affect brain amines. The antidepressant action of DMI becomes dramatically evident in studies which show that in rats pretreated with the drug the effects of RO-4-1284 or reserpine are reversed. The action of the drug in this regard is far more rapid and potent than that of imipramine. In fact imipramine itself does not seem to have this action for it actually inhibits the action of DMI. Preliminary clinical studies in collaboration with N. KLINE (unpublished) indicate that DMI is potent and rapidly acting in endogenous depression.

Zusammenfassung. Nach Verabreichung von Imipramin (Tofranil) wurde aus dem Gehirn von Ratten Desmethylimipramin, das N-Monomethylderivat, isoliert. Dieser Metabolit beziehungsweise dessen Anreicherung *in vivo* scheint für die antidepressive Wirkung von Imipramin verantwortlich zu sein.

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Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda (Maryland), July 17, 1961.

[7] We thank Dr. W. J. A. VAN DEN HEUVEL for performing the gas chromatography analyses.

[8] B. HERRMANN and R. PULVER, Arch. int. Pharmacodyn. *126*, 454 (1960). Reference samples of desmethylimipramine and desdimethylimipramine were obtained through the courtesy of Dr. F. HAEFLIGER, J. R. Geigy, S.A., Basel.

[9] B. HERRMANN, W. SCHINDLER, and R. PULVER, Med. exp. *1*, 381 (1959).

[10] F. SULSER, J. V. DINGELL, J. R. GILLETTE, and B. B. BRODIE, in preparation.

Biophysikalische Studien mit synthetischem Lecithin als Weg zu neuartigen Chemotherapeutika

In unserer letzten Mitteilung[1] haben wir dargelegt, dass dem Lecithin vermutlich die biologische Funktion eines «carriers» beim Ionentransport durch den lipoiden Teil der Zellwand zukommt.

In einem einfachen biophysikalischen Modell konnten wir zeigen, dass Lecithin, gelöst in CCl_4, befähigt ist, Anionen aus wässriger Lösung in die lipoide Phase überzuführen, wenn in der wässrigen Phase zugleich gewisse Kationen vorhanden sind. Aus messtechnischen Gründen wurde als Anion Tropäolin verwendet. Die Bindungsfähigkeit zwischen Lecithin und diesem Farbstoff ist stark abhängig von der Art und Konzentration der anwesenden Kationen. Es wurde gefunden, dass insbesondere polybasische Verbindungen wie Protamin und Polymyxin den Transport in die lipoide Phase fördern.

In weiteren Versuchen stellten wir fest, dass in CCl_4 gelöstes Lecithin befähigt ist, in Wasser gelöstes Trypaflavin (Kation) in die lipoide Phase überzuführen und dass dieselben Kationen, die den Übergang von Tropäolin fördern,

[1] R. HIRT und R. BERCHTOLD, Med. exp. *2*, 269 (1960).