Different tissues gave different degrees of fluorescence and homologous systems usually gave a greater degree of fluorescence than interspecies reactions. This might be explained on the basis of slight differences in antigenic relation, such as those within various virus strains when cultivated in different mammalian tissues; or, might be attributed to differences in the viruses, notwithstanding antigenic similarities.

Zusammenfassung. Antikörper bei genesenden Mäusen nach Infektion mit Leukämie-Viren (S-63) erzeugt, zeigen eine Kreuzreaktion mit den Leukämie-Antigenen aus Rinder- und Menschenserum und Gewebe. Diese Befunde sprechen dafür, dass die Viren-Antigene in den drei Systemen sehr ähnlich sind.

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The Conversion of H^3-Tyrosine to H^3-DOPA in the Adrenal Glands under in vivo Conditions

Recently UDENFRIEND et al.[1-3] were able to demonstrate the formation of DOPA by incubating tissue slices of adrenals and homogenates of adrenal medulla and of brain with radioactive tyrosine. Furthermore they could show the DOPA formation by an in vitro system with purified tyrosine hydroxylase. This paper demonstrates the synthesis of DOPA under in vivo conditions.

Domestic fowl and cats were injected with H^3_2-3,5-L-tyrosine (30,000 mc/mM)[4], 100 mc/kg, intravenously in urethan (1 g/kg) and ether anaesthesis, respectively. The animals were decapitated 20 min after injection. The DOPA decarboxylase was inhibited with 100 mg/kg NSD-1034 (N-methyl-N-(3-hydroxybenzyl)hydrazine dihydrogen phosphate)[5] in some of the animals by i.v. injection 20 min before tyrosine injection. About 1 g of various organs (adrenal glands, brain stem, heart, liver, kidney, spleen, pancreas and blood) were homogenized with 4 ml water, to which was added 1 mg each of carrier tyrosine, DOPA, dopamine, norepinephrine, epinephrine and glutamic acid. The acid soluble fraction was isolated with trichloroacetic acid according to the SCHMIDT-THANNHAUSER method[6]. The isolation of H^3-DOPA was carried out in the following way: After extraction of the trichloroacetic acid with ethyl acetate ester the acid soluble compounds were fractionated by high-voltage paper electrophoresis (buffer: pyridine/glacial acetic acid/water, 4:1:47 v/v, pH 5.1, field-strength 40 V/cm, $t = -8$ °C, 180 min; paper: Schleicher and Schüll 2043 b Mgl., washed). The tyrosine-DOPA-spot was eluated and descending chromatography was carried out in n-butanol/glacial acetic acid/water (4:1:5, organic phase, SO_2-atmosphere; paper: Schleicher and Schüll 2043 b Mgl.). This system separated tyrosine from DOPA. The DOPA-spot was eluated again, and DOPA was characterized by its complex with boric acid in high-voltage paper electrophoresis (buffer: boric acid/sodium hydroxide/water, 155:16:5 g/g/l, pH 8.0, field-strength 80 V/cm, $t = -8$ °C, 60 min; paper: Schleicher and Schüll 2043 b Mgl.). With this procedure it was possible to recover 74.3 ± 3.6% of added H^3-5-L-DOPA. All the solutions were prepared with oxygen-free water. Radioactivity was measured by liquid scintillation counting (Tri-Carb, EX 314)[7,8]. In experiments with cats, specimens of effluent venous blood from the adrenals were taken at different times after H^3-tyrosine injection. This was performed by preparing a sac from v. cava inferior, into which only venous blood from the adrenals emptied. All other veins of this region were ligatured. Details of the methods will be published elsewhere.

The Table shows the content of H^3-DOPA in the acid soluble fraction of adrenals and brain stem of domestic fowl and of cats 20 min after i.v. injection of H^3_2-3,5-L-tyrosine. Radioactive DOPA could be found in the adrenals after inhibition of the DOPA decarboxylase by NSD-1034. In domestic fowl H^3-DOPA represented 3.95% and in cats 5.09% of the total radioactivity. Since H^3_2-3,5-L-tyrosine loses 50% of its tritium label during conversion into H^3-5-L-DOPA, the content of labelled DOPA would be approximately twice as large as in the Table if a tyrosine labelled at a different position were used. Neither in the brain stem nor in any other organ investigated, could radioactive DOPA be detected, not even after inhibition of DOPA decarboxylase. Large amounts of radioactive catecholamines were isolated from adrenals in experiments without NSD-1034. The major part (about 80%) of these catecholamines was H^3-dopamine. The H^3-catecholamine content in adrenals of domestic fowl was significantly higher than in cats. This is explained by the higher percentage of chromaffine tissue in adrenals of fowl[9]. H^3-tyramine was absent from all organs examined (< 0.005 μg/g wet tissue)[10].

The Figure presents the concentrations of H^3-tyrosine and H^3-DOPA (μc/ml) in the venous blood of the adrenals of cat between 1 and 18 min after i.v. injection of radioactive tyrosine. Most of the DOPA formed in the adrenals

[1] T. NAGATSU, M. LEVITT, and S. UDENFRIEND, Biochem. biophys. Research Commun. *14*, 543 (1964).
[2] T. NAGATSU, M. LEVITT, and S. UDENFRIEND, J. biol. Chem. *239*, 2910 (1964).
[3] S. UDENFRIEND, P. ZALTZMAN-NIRENBERG, and T. NAGATSU, Biochem. Pharmac. *14*, 837 (1965).
[4] L. BIRKOFER and K. HEMPEL, Chem. Ber. *96*, 1373 (1963).
[5] The supply of NSD-1034 from Smith and Nephew Research Ltd., Harlow, England, is gratefully acknowledged.
[6] G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. *161*, 83 (1945).
[7] F. KALBERER and J. RUTSCHMANN, Helv. chim. Acta, *64*, 1956 (1961).
[8] K. HEMPEL, Atompraxis *10*, 148 (1964).
[9] D. M. SHEPHERD and G. B. WEST, Br. J. Pharmac. *6*, 665 (1951).
[10] K. HEMPEL and H. F. K. MÄNNL, Naunyn-Schmiedebergs Arch.

was secreted into the venous blood, and only about 5% of the total amount was detected in the gland at the end of the experiments. Contrary to this result, we failed to detect H^3-DOPA in the blood from any other organ.

Though some authors[11–13] could find DOPA in adrenal glands, other investigators failed to detect it[9,14,15]. On the other hand, the occurrence of DOPA in phaeochromocytoma tissue[1,6,7] and in urine of patients with neuroblastoma[18–20] could be ascertained. Even by using highly sensitive radioactive methods, we could detect DOPA in adrenals only after inhibition of the DOPA decarboxylase. NAGATSU et al.[2] measured the tyrosine hydroxylase activity in the brain stem, which was in the same range as in the adrenal glands. Contrary to this result we failed to demonstrate the DOPA formation in brain stem under in vivo conditions. ANTON et al.[20] were also unsuccessful in finding DOPA in the brain stem by a fluorometric method.

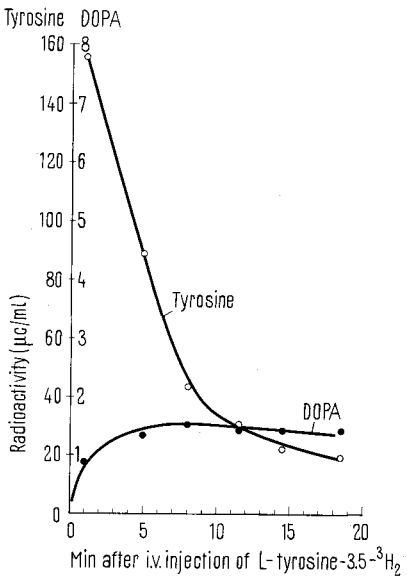
Besides its occurrence in chromaffine tissue, DOPA is an intermediate in melanin biosynthesis[21–24]. Under in vivo conditions we recently succeeded in demonstrating the DOPA formation from tyrosine in mouse melanomas with a method similar to that described in this paper[25].

No appreciable amount of DOPA can be stored in the medulla, because more than 90% of the H^3-DOPA was detected in the venous blood of the gland. This result shows that the catechol configuration is not of chief importance in the binding of catecholamines, as already suggested by WEINER and JARDETZKY[26]. The quantity of H^3-DOPA (DOPA-effluent + DOPA in the gland) formed in the adrenals of animals treated with NSD-1034 was in the same range as the quantity of H^3-catecholamines formed without DOPA decarboxylase inhibition. This indicates that NSD-1034 does not influence the tyrosine hydroxylase activity[27].

Content of H^3-DOPA 20 min after i.v. injection of H^3-tyrosine

	NSD-1034 inhibition	Organs	% of total radioactivity[a]		
			Tyrosine	Catechol-amines	DOPA
Domestic fowl	–	Adrenals	34.9	49.8	0.17
		Brain stem	80.2	0.3	0.07
	+	Adrenals	82.1	0.3	3.95
		Brain stem	92.2	0.01	0.15
Cat	–	Adrenals	46.8	24.7	0.53
		Brain stem	95.0	2.8	0.29
	+	Adrenals	93.9	0.01	5.09
		Brain stem	97.0	0.00	0.49

[a] Mean values from 2 animals. The total radioactivity of the acid-soluble fraction after extraction of the trichloroacetic acid with ethyl acetate ester and evaporating to dryness was put at 100%.



Concentrations of H^3-tyrosine and H^3-DOPA in the venous blood of the adrenals of cat as a function of time after i.v. injection of 100 mc/kg H^3_2-3,5-L-tyrosine. Inhibition of DOPA decarboxylase with NSD-1034 (100 mg/kg).

Zusammenfassung. Nach i.v. Injektion von H^3-Tyrosin und Blockung der DOPA-Dekarboxylase wurde H^3-DOPA in den Nebennieren von Hähnchen und Katzen mit Hilfe einer Kombination von papierhochspannungselektrophoretischen und chromatographischen Methoden nachgewiesen. In anderen Organen war dagegen H^3-DOPA nicht nachweisbar. Bei den Katzen konnte gezeigt werden, dass H^3-DOPA in den Nebennieren nicht gespeichert, sondern zum weitaus überwiegenden Teil an das venöse Blut abgegeben wird.

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exp. Path. Pharmak., in press.
[11] McC. GOODALL, Acta chem. Scand. *4*, 550 (1950).
[12] E. WERLE and J. JÜNTGEN-SELL, Biochem. Z. *327*, 259 (1955).
[13] J. B. BOYLEN and J. H. QUASTEL, Biochem. J. *80*, 644 (1961).
[14] G. ROSENFELD, L. C. LEEPER, and S. UDENFRIEND, Arch. biochem. Biophys. *74*, 252 (1958).
[15] G. HALL, N.-A. HILLARP, and G. THIEME, Acta physiol. scand. *52*, 49 (1961).
[16] H. WEIL-MALHERBE, Lancet *282* (1956).
[17] B. E. CABANA, J. C. PROKESCH, and G. S. CHRISTIANSEN, Arch. biochem. Biophys. *106*, 123 (1964).
[18] W. VON STUDNITZ, Scand. J. clin. Lab. Invest. *12*, suppl. 48 (1960).
[19] W. VON STUDNITZ, H. KÄSER, and A. SJOERDSMA, New Engl. J. Med. *269*, 232 (1963).
[20] A. H. ANTON and D. F. SAYRE, J. Pharmac. exp. Ther. *145*, 326 (1964).
[21] H. BERNHEIMER, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *246*, 12 (1963).
[22] J. A. SCOTT, Lancet *861* (1962).
[23] A. B. LERNER, T. B. FITZPATRICK, E. CALKINS, and W. H. SUMMERSON, J. biol. Chem. *178*, 185 (1949).
[24] M. FOSTER and S. R. BROWN, J. biol. Chem. *225*, 247 (1957).
[25] K. HEMPEL and H. F. K. MÄNNL, Biochim. biophys. Acta, in press.
[26] N. WEINER and O. JARDETZKY, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *248*, 308 (1964).
[27] This work was supported by the Deutsche Forschungsgemeinschaft.