of reserpine, the fusimotor activity disappeared simultaneously with the appearance of the rigidity in the emg (Figure 1).

The spinal reflexes were studied by stimulation of the dorsal root of the 4th caudal segment and recording from the corresponding ventral root in rats lightly anaesthetized with Viadril (Pfizer) and nitrous oxide. Fusimotor activity in the ventral root and the gastrocnemius electromyogram were also recorded. After the reserpine injection, the monosynaptic reflexes were strongly increased at the same time that rigidity appeared and fusimotor activity disappeared (Figure 2)[9].

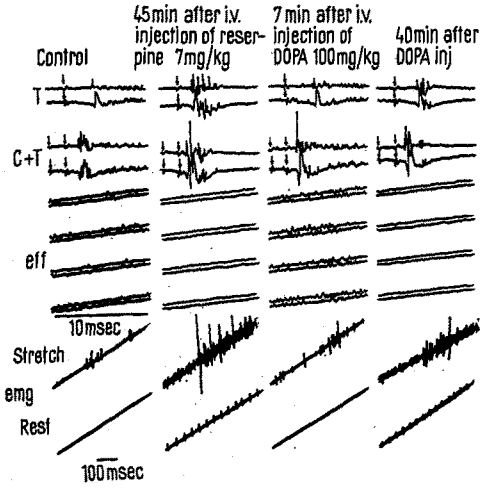

Fig. 2. The reflexes were recorded using two pairs of recording electrodes 9 mm apart on a coccygeal ventral root. The dorsal root was stimulated with a single test pulse (T) and with a conditioning pulse preceding the test pulse (C + T). Shock artefacts indicated by arrows. The spontaneous efferent discharge was also recorded from the ventral root (eff). Reserpine increased the monosynaptic reflex, abolished the fusimotor activity and induced rigidity. DOPA reversed the effects of reserpine.

In order to test whether the results had bearing upon the clinical state of Parkinsonan, DOPA (100 mg/kg) was injected i.v. in the reserpine rats. It caused a reversible disappearance of the rigidity, reappearance of the fusimotor activity and reduction of the monosynaptic reflexes[10].

Reserpine thus causes a shift from γ- to α-efferent-activity simultaneously with the appearance of rigidity. The similar effects of DOPA on reserpine rigidity in rats and Parkinsonian rigidity support the assumption that the results gained in the rat experiments reflect the pathophysiology of Parkinsonism.

All factors discussed above connect the Parkinsonian and the reserpine rigidity and indicate an α-rigidity in Parkinsonism.

Zusammenfassung. Ein parkinsonähnliches Syndrom von Akinesie, Rigidität und Tremor wurde in Ratten durch Reserpin induziert. Gleichzeitig mit dem Auftreten der Rigidität wurde die spontane Aktivität der einzelnen γ-Efferenen zum Stillstand gebracht, weil die monosynaptischen Reflexe gesteigert geworden waren. Das reserpininduzierte akinetische Syndrom stellt also eine α-Rigidität dar.

Die Ähnlichkeit der Wirkung von L-DOPA auf das reserpininduzierte Syndrom und das Parkinsonsyndrom bei Menschen lässt eine gemeinsame Pathophysiologie vermuten.

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Mast Cells and Monoamines

Studies on the human skin suggest that, besides the adrenergic nerves, there exist local tissue stores of some monoamine which may be a vasoconstrictor, and that these stores may be represented by chromaffin cells[1–5]. The cells may belong to the group known as 'tissue mast cells'[6,7]. The existence of chromaffin cells in the human skin has been questioned[8].

With the use of highly sensitive and specific fluorescence methods[9–13] for the direct demonstration of monoamines and some of their precursors (e.g. L-dopa) at the cellular level, we have started experiments to elucidate the existence and significance of local monoamine stores in the tissues – especially the skin – of mammals. Some of the results obtained are reported briefly in this paper. Unless otherwise stated, the tissues were freeze-dried, treated with formaldehyde gas, embedded in paraffin, sectioned and mounted for fluorescence microscopy according to the formaldehyde method[10–12]. Catecholamines and 5-hydroxytryptamine (5-HT) are in this way converted to intensely fluorescent 3,4-dihydroisoquinolines (green) and a 3,4-dihydro-β-carboline (yellow), respectively[13].

Only two monoamine-storing structures have been found so far in the skin of mouse, rat, guinea-pig, hamster, rabbit and cat: (1) Adrenergic nerves, which have in their terminals very high concentrations of noradrenaline[11], and (2) specific cells that on the basis of their staining with Astrablau (at pH 0.2 according to BLOOM and

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KELLY[14]) and their strong metachromasia (toluidine blue staining) must be classified as mast cells. In *mouse* and *rat*, most if not all of the mast cells develop an intense yellow fluorescence, which is no doubt due to 5-HT[11,13] well known to be present in high concentrations in these cells. Most of the mast cells in *hamster* and *rabbit* skin, on the other hand, show a moderate and weak to very weak green fluorescence, respectively. The characteristics of the fluorescence reaction and the properties of the fluorescent products[11,13,15] strongly support the view that these cells contain a primary catecholamine. No evidence for the presence of adrenaline was obtained in experiments with another fluorescence method[9]. The fact that the fluorescence reaction is very markedly reduced 24 h after administration of reserpine (25 and 5 mg/kg, respectively) is further evidence that it is due to some monoamine.

After the administration of L-dopa (50 mg/kg subcutaneously), the mast cells that in normal *rabbits* only fluoresce weakly develop an intense and brilliant green (to yellow-green) fluorescence (Figure 1). It could then be clearly seen that the fluorescent products, which on the basis of the histochemical criteria must derive from dopa itself or from primary catecholamines, are localized to the cytoplasmic granules (Figure 4). It was then easier also to identify the cells as mast cells. (The sections were first photographed in the fluorescence microscope, then stained with Astrablau or toluidine blue and photographed again. The increase in fluorescence observed after dopa administration became marked after 30 min, reached a maximum within 2 h and then showed no definite changes for 24 h. A fairly marked increase was observed already after a dose of 5 mg/kg. A similar increase was produced also by D-dopa but at a ten times higher dose level. The administration of dopamine (75 mg/kg subcutaneously, 30 min to 6 h), on the other hand, caused no increase in fluorescence. If reserpine (5 mg/kg i.v.) was given 2 to 12 h before the injection of dopa, no or only a slight increase was observed 4 to 12 h later. Inversely, the intense fluorescence that develops after dopa administration was very markedly reduced and sometimes practically abolished 8 to 9 h after the injection of reserpine. If the potent dopa decarboxylase inhibitor NSD 1015[16,17] (100 mg/kg intraperitoneally) was given 1 h before dopa and the animals were killed 1 h later, it prevented the increase in fluorescence intensity otherwise observed. This effect was partially overcome if the dose of dopa was increased to 100 mg/kg and the animals were killed 2 h later.

The mast cells in *hamster* skin were examined in a similar way. All the mast cells that normally showed a distinct fluorescence developed a very much stronger fluorescence after the administration of dopa (Figures 2 and 3).

The amine metabolizing capacity of the specific mast cells is thus clearly demonstrated. The results obtained give further support to the view that they normally contain low concentrations of a primary catecholamine. There is little doubt that the cells are able to take up large amounts of L-dopa, but not dopamine. The experiments with reserpine and NSD 1015 indicate that the dopa taken up cannot be retained by the cells but is rapidly decarboxylated to primary amines which can be retained and stored in high concentrations in the cytoplasmic granules. Reserpine both blocks this amine storage and depletes the stores formed before its administration.

The cells thus seem to have the specific characteristics of monoamine-forming cells which can take up L-dopa, contain dopa decarboxylase and have an amine storage mechanism that is blocked by reserpine. None of the findings obtained so far conflict with this view, and we hope that further evidence will be forthcoming in the continued experiments.

The mast cells in *cat* skin show a very weak green fluorescence or none at all. The fluorescence was not observed in reserpinized animals (5 mg/kg, 24 h). After administration of L-dopa (100 mg/kg subcutaneously, 2 to 6 h) many of the mast cells exhibited a strong green fluorescence (not

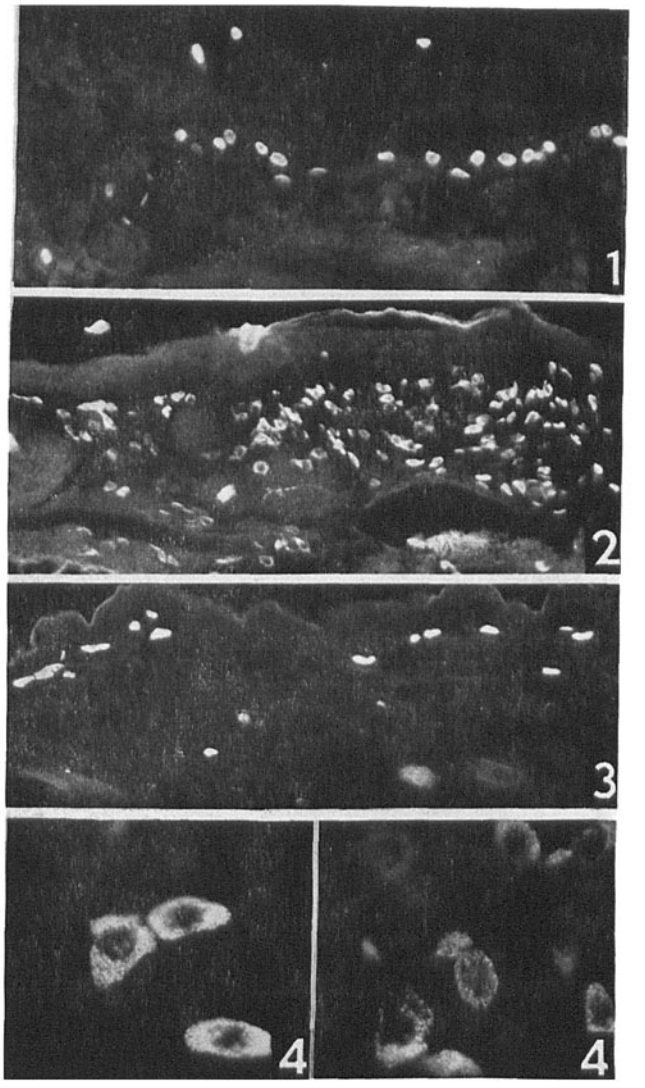

Fig. 1. Skin of rabbit ear 24 h after administration of L-dopa (50 mg/kg). Strongly fluorescent mast cells. × 165.

Fig. 2. Skin of hamster ear 1 h after administration of L-dopa. Abundant mast cells with strong fluorescence. × 165.

Fig. 3. Skin of hamster hind foot 7 h after administration of L-dopa. × 165.

Fig. 4. Skin of hamster ear 4 h after administration of L-dopa. × 660.

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Tissues	Rat (4)		Guinea-pig (8)		Hamster (7)		Rabbit (4)		Cat (3)	
	1	2	1	2	1	2	1	2	1	2
Skin, ear	2+	100	2+	0	4+	50–75	2+	80– 90	4+	5– 10
Skin, feet			1+	0	2+	40–50	1+	70– 80	3+	30– 50
Skin, nose			2+	0	2+	30–50	1+	90–100	2+	80– 90
Skin, abdomen			1+	0	2+	5–15	1+	90–100	2+	10– 20
Skin, thigh			1+	0	2+	5–15	1+	80– 90	2+	10– 20
Soles of the feet			2+	0	1+	30–50	1+	80– 90		
Tongue	3+	100	1+	0	3+	0	Few	100	2+	90–100
Oesophagus	2+	100	1+	0	2+	0			2+	20– 30
Inf. nasal concha			1+	0			Few	0	1+	90–100
Cheek pouch					3+	0				

1. Relative mast cell content (staining with Astrablau). 2. Number of mast cells (in % of the total number) which became strongly fluorescent 2 to 4 h after administration of L-dopa (rabbit 50 mg/kg, the others 100 mg/kg). The rats were not treated with dopa. The figures in parentheses are the number of animals examined.

so intense as that in rabbits treated with lower doses of dopa). In *guinea-pig*, on the other hand, all the mast cells in the skin were non-fluorescent even after this large dose of dopa.

The mast cells in the outer layers of the ear skin of *hamster* are all practically of the specific type described above. However, most of the cells in the deepest layers do not develop any fluorescence at all – even after the administration of a large dose of dopa (100 mg/kg). Both types are present in other skin areas and the amine cells are usually located in the subepidermal tissue. In some tissues of the hamster, the picture is quite different. In the mucosa of the cheek pouch, tongue and oesophagus, there are a great number of mast cells, all of which belong to the type which does not develop fluorescence even after dopa administration.

The group classified as 'mast cells' on the basis of the staining properties of their granules, thus seems to contain two distinctly different types of cells. This view is strongly supported by the findings obtained when closer examinations were made of the mast cell population in different skin areas and some other tissues (Table). Both types were readily observed in cat and hamster but, as seen in the Table, the two species differ as regards the localization of the amine cells. In rabbit most of the mast cells have so far been found to belong to this category. In contrast to other animals, the guinea-pig does not seem to have any mast cells of the amine type at all. All of the Astrablau-positive cells in the rat tissues examined before the administration of dopa, on the other hand, showed an intense fluorescence which no doubt was due to 5-HT. In this connection it is of great interest that the new type of chromaffin cells storing dopamine, discovered[18] some years ago in the tissues of ruminants, has now also been definitely proved[19] to belong to the mast cell group. No closer examination of the mast cell population in these species has as yet been made.

There is thus good evidence that, with one exception, all mammalian species so far examined with adequate methods have local tissue stores of monoamines in a system of specific cells which belong to the large group known as 'tissue mast cells'. These cells seem to have the specific characteristics of monoamine-forming cells. In three of the four species closely examined with direct methods in the present work, this cell system has been found to constitute only a part of the mast cells. On the other hand, no amine-forming mast cells have so far been detected in the guinea-pig. From a biochemical point of view, the tissue mast cells thus seem to be a heterogeneous group, containing at least two distinctly different cell types. Electron microscopic observations on human mast cells have previously revealed two types of cells with different morphology[6,7].

The specific mast cells in ruminants (chromaffin) and rat have high concentrations of dopamine and 5-HT, respectively. In hamster they seem to contain fairly large amounts of a primary catecholamine. In rabbit and cat, on the other hand, they seem to have a low content of some amine belonging to this category but, in spite of this, have a high capacity to store some monoamine formed in them from L-dopa. We are therefore working with the hypothesis that the amines demonstrated in the untreated animals are intermediate metabolites and that the final product normally stored in the cells is a catecholamine which cannot condense with formaldehyde but may form dark pigments on oxidation with bichromate (see the introduction). With the use of model systems, we have found (unpublished data) that catecholamines which are tertiary amines fulfill both these requirements. Chemical determinations will be made to test the hypothesis[20].

Zusammenfassung. Mit einer Ausnahme scheinen alle Arten von Säugetieren, die mit vergleichbaren Methoden untersucht worden sind, lokale Gewebslager von Monoaminen zu haben in einem System von spezifischen Zellen, die zur grossen Gruppe der Gewebsmastzellen gehören. Diese Zellen scheinen die spezifischen Merkmale der monoaminbildenden Zellen zu besitzen. Biochemisch scheinen die Gewebsmastzellen an einer heterogenen Gruppe zu gehören, die aus wenigstens zwei ganz verschiedenen Zelltypen besteht.

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[20] *Acknowledgments.* This work has been supported by research grants from the United States Public Health Service (NB02854-04), Stiftelsen G. och T. Svenssons Minne and by the United States Air Force under Grant No. AF 61(052)-450.