peroxidase[8] – represent pure high-spin complexes[18]. In contrast to this, catalase cyanide shows a typical low-spin spectrum with the g-factors $g_1 = 2.78$, $g_2 = 2.15$, $g_3 = 1.60$ (Figure 2). The magnetic titration of the catalase with potassium cyanide made by THEORELL and AGNER[1] showed that only $^3/_4$ of the catalase iron was cyan-sensitive, while the last $^1/_4$, the non-hem iron, was not affected by cyanide. Accordingly, the ESR spectrum of the catalase cyanide shows that the total porphyrin-bound iron enters a binding with cyanide. The iron which is not porphyrin-bound, having a g-value of 4.2, does not react with cyanide, that is the absorption at $g = 4.2$ does not change upon reaction of catalase with potassium cyanide. The ESR spectrum of catalase azide indicates that this compound must be a mixed complex of the high-spin and low-spin form similarly as methemoglobin and some methemoglobin compounds[9], the peroxidase cyanid[8] and the cytochrome c peroxydase[12].

The g-values of catalase azide are $g_\perp \approx 6.5$ and for $g_1 = 2.66$, $g_2 = 2.6$, $g_3 = 1.7$.

Since the measurement of the magnetic susceptibility for the catalase azide complex gave a high value at 20 °C which corresponds to 5 unpaired electrons[1], it must be assumed that at low temperature the low-spin proportion increases as was also observed for methemoglobin[19].

If catalase azide is allowed to react with hydrogen peroxide or barium peroxide without oxygen and is then frozen immediately after the reaction, then the ESR measurement of this frozen solution gives a spectrum in which the absorption at $g \approx 6$ is strongly reduced compared with the catalase azide spectrum. At the same time an intensive narrow signal occurs at $g = 2$. This signal can be attributed either to a free radical or to ferro-catalase-nitric oxide which is found by FOULKES and LEMBERG[20] and by NICHOLLS[21] as a product of the reaction from catalase azide with hydrogen peroxide. Ferro-catalase-nitric oxide will give a narrow ESR signal of the same widths as a free radical like hemoglobin-nitric oxide[22].

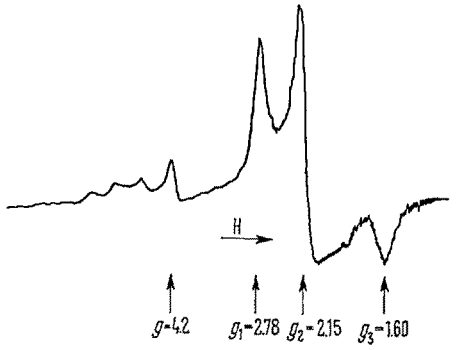

Fig. 2. First derivative of electron spin resonance absorption spectrum from catalase cyanide.

Free radicals have also been observed in the reaction of hydrogen peroxide with other hemoproteids[8,12,23]. This finding is of interest because THEORELL and EHRENBERG[3] have found that the azide catalase-H_2O_2-complex in nitrogen or CO atmosphere is a diamagnetic complex from which they conclude that a ferro complex has formed. However, the presence of a free radical or ferro-catalase-nitric oxide by the reaction of hydrogen peroxide with catalase azide indicates that a complete diamagnetism cannot be possible. Is it possible that both, a free radical on the protein and ferrocatalase-nitric oxide, are present in the compound formed on the addition of catalase azide with hydrogen peroxide in nitrogen.

ESR studies of the reaction of cytochrome c peroxidase with ethyl hydroperoxide, in which the ESR absorption of the porphyrin-bound iron disappears with simultaneous formation of a free radical[12], suggest that the present ideas about the process of azide catalase hydrogen peroxide reaction require further investigations.

Zusammenfassung. Rinderleberkatalase und einige Katalasekomplexe wurden bei einer Temperatur von 77 °Kelvin mit der Methode der Elektronenspinresonanz untersucht. Aus den Elektronenspinresonanzspektren ist zu entnehmen, ob es sich bei der Katalaseverbindung um einen Gross-spin-Komplex, um einen Klein-spin-Komplex oder um eine Mischform beider Komplexarten handelt.

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Striatal Bradykinesia Alleviated by Intracaudate Injection of L-Dopa

In former experiments it was shown[1] that prolonged chemical stimulation of the caudate nucleus in cats by injection of alumina cream into this ganglion induces first a bradykinesia and later a nearly complete akinesia and a catatonia-like condition. The chemical stimulation of the caudate nucleus seemed useful for a study of the effect of L-dopa, which is converted into dopamine in the tissues, upon this ganglion. Such a study seemed of interest in view of the findings that the dopamine content of the caudate nucleus is low in Parkinson's disease[2], and that therapeutic administration of L-dopa is able to relieve some symptoms of this disease, particularly the akinesia, at least temporarily.

Experimental. Since it takes several days until the effect of the alumina cream injections becomes obvious,

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Effect of intracaudate injections upon speed of locomotion

Tungstic acid effect (speed decrease)			Dopa effect (speed increase)	
Series 1 N = 13	Speed before minus speed after injection	Mean difference 2.56 ± 0.92[a] $t = 10$ $p < 0.001$	Speed after minus speed before injection	Mean difference 2.35 ± 1.37 $t = 6.2$ $p < 0.001$
Tungstic acid effect			Effect of solvent without dopa (control)	
Series 2 N = 10	Speed before minus speed after injection	1.93 ± 0.95 $t = 6.4$ $p < 0.001$	Speed after minus speed before injection	0.3 ± 0.89 $t = 1.1$ not significant

[a] Figures indicate number of rotations of pulley. One rotation corresponds to a locomotion of 8.5 cm on floor. t, mean difference divided by the standard error of the mean.

and the catatonia becomes so severe that it can no longer be influenced, a faster and milder method of chemical stimulation was tried. In preliminary experiments carbachol[3] was injected into the caudate nucleus of cats. For testing, the animals were suspended in a hammock, with the extremities passing through its holes. One hind leg was kept between an electric bulb and a photocell; the output of the latter was recorded; it fluctuated when movements of the limb interrupted the light beam. Although by this method a return or increase of motility in akinetic or bradykinetic animals could be demonstrated following intracaudate L-dopa injections[4], the records did not always give a complete picture of the leg movements, since these movements fell sometimes outside the path of the light beam to the photocell.

A different technique was, therefore, applied in the present series of experiments. In 33 animals the anatomic examination revealed a correct localization of the injections in albino rats. Tungstic acid gel previously used by BLUM and LIBAN[5] for production of irritative foci in the hippocampus and amygdala proved very suitable. Injection of 4–8 μl into the caudate nucleus was sufficient to induce a definite slowing of the animals' locomotion. In order to record the running speed (spontaneous locomotion or that induced by acoustic or nociceptive stimuli), a thread was conducted from the animal over a lower and an upper pulley to a small weight. With each rotation of the upper pulley, the discharge of a photocell was activated by a light beam passing through a hole in the upper pulley. Each discharge indicated a movement of 8.5 cm on the floor. When the slowing was definite, 4–8 μl of L-dopa solution (pH = 5) containing 80–160 μg of L-dopa were injected into the caudate nucleus. In control experiments, corresponding amounts of physiologic saline solution or of the solvent (HCl solution neutralized to pH 5) were injected into the caudate nucleus.

Results. The main results are summarized in the Table. They show that the intracaudate injection of tungstic acid gel induced a statistically highly significant slowing of the rat's locomotion. In further (non-tabulated) experiments it was found that the slowing persisted up to 20 h, if no other treatment was applied. Injection of L-dopa into the caudate nucleus in untreated rats increased their running speed for about $1^1/_2$ h. Intracaudate injection of this substance 1 h after the tungstic acid injection, when the slowing was definite, resulted in a statistically significant increase of the running speed within a few min (Table). However, if the tungstic acid gel had produced not only a bradykinesia, but a cataleptic state (complete akinesia), the L-dopa was only able to produce short lived, incoordinated rapid movements, but not a definite running movement. In control experiments (Table, series 2), injection of the solvent or of NaCl in amounts corresponding to the L-dopa injections failed to alter significantly the bradykinesia, or this even progressed, so that the L-dopa effect can not be attributed to the mechanical injury due to the injection.

Discussion. In agreement with the above described findings that L-dopa counteracts the effects of chemical stimulation of the caudate nucleus are electrophysiologic studies; macroelectrode[4,6] as well as microelectrode recordings[7,8] indicate that L-dopa application chiefly depresses the electric activity of the caudate nucleus. It should be emphasized, however, that the striatal effect is not necessarily the only mechanism responsible for the beneficial action of L-dopa observed in Parkinson patients, since decarboxylase converting dopa into dopamine is found also in other parts of the central nervous system[9].

Conclusions. The bradykinesia resulting from chemical stimulation of the caudate nucleus can be alleviated by injection of L-dopa into the ganglion[10].

Zusammenfassung. Die durch chemische Reizung des Nucleus caudatus herabgesetzte Laufgeschwindigkeit konnte durch Injektion von L-Dopa in dieses Ganglion wieder den ursprünglichen Werten nahegebracht werden.

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