After dialysis the material of each of the 3 peaks obtained was concentrated and tested for chemotactic activity as previously described[7]. The material of the first and second peak had no chemotactic activity, whereas the material of the third peak induced pronounced migration. Both halves of the third peak were equally active (Figure 2). It has been shown that there is a good correlation between molecular weight and elution volume from Sephadex columns[8]. FLODIN and KILLANDER[9] have demonstrated that, by filtration of serum through a Sephadex G-200 column, 3 peaks are obtained, the first peak consisting of 19*s*–7*s* material, the second peak of 7*s*–4*s* and the third peak of 4*s* and smaller material. This suggests that the cytotaxins recovered in the third peak consist of 4*s* or smaller material. NILSSON and MUELLER-EBERHARD[10] have however presented evidence that the complex of the complement components C'5 and 6 which has been identified as cytotaxin[11] has a sedimentation coefficient higher than 9.5*s*. This complex would presumably be eluted in the first peak, yet in our experiments no chemotactic activity was detected in this fraction. This could be due to the fact that in the present experiments separations have been performed at slightly alkaline pH, where the C'5, 6 and 7 complex dissociates and is thereby inactivated[10].

Activity could however be detected in the first peak under slightly different experimental conditions. 2 fractions were obtained from chemotactic serum by ammonium sulphate precipitation: fraction 1: 0–0.33 saturation; fraction 2: 0.33–0.7 saturation. Both fractions were found to be chemotactic. After chromatography of fraction 1 on Sephadex G-200 chemotactic activity was detected in the first peak. A similar chromatographic separation of fraction 2 showed activity in the third peak only, the first and second peaks being inactive. It is possible that the activity in the first peak is due to a C'5, 6 and 7 complex. Again the chemotactic activity found in the third peak cannot be attributed to this complex. Consequently, the presence of more than one polymorph cytotaxin in serum must be considered. This view could explain the apparent discrepancies between our previous findings that formation of chemotactic activity does not parallel formation of hemolytic complement[6] and those of WARD, COCHRANE and MUELLER-EBERHARD[5] that the chemotactic activity in serum is due to C'5, 6, 7 complexes.

It has been shown that the cytotaxins formed in fresh serum on interaction with antigen-antibody mixtures are specific to polymorphs. In a first series of experiments, serum exerted a slight effect on mononuclear cells; but it was not clear whether this was an unspecific response or whether small quantities of a macrophage cytotaxin[3] were present. Further experiments with different pools of normal rabbit serum have however shown that such sera contain varying and sometimes considerable chemotactic activity for mononuclear cells (Table). The pronounced effect observed with certain sera suggests the presence of a cytotaxin for mononuclear cells. The mechanism of its formation remains unknown.

The data presented give evidence for the occurrence of several cytotaxins in rabbit serum. At least 2 different cytotaxins for polymorphs and 1 cytotaxin for mononuclear cells have been demonstrated[12,13].

Zusammenfassung. Die Untersuchungen zeigen, dass mehrere Zytotaxine im Kaninchenserum vorhanden sein können. Zwei davon wirken spezifisch auf Granulozyten und ein anderes spezifisch auf mononukleare Zellen.

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Chemotactic activity for mononuclear cells in normal rabbit serum

Test solution	Mononuclear cells/field
Gey's solution	0
Casein (10 mg/ml Gey's solution) (positive control)	292
20% normal rabbit serum in Gey's solution	177

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Presynaptic Inhibition by Gastrocnemius-Soleus Nerves

Synchronous excitation of Group I afferent fibres of nerves to flexor muscles of cat hind limb induces presynaptic inhibition of the monosynaptic reflex in response to stimulation of either flexor or extensor nerves[1,2]. No evident presynaptic inhibition was found following the electrical stimulation at Group I strength of the nerves to the extensor muscles of hip, ankle and toe[1,2]. However, afferent volleys following the contraction of an extensor muscle, such as the gastrocnemius medialis (MG), cause presynaptic inhibition of Ia afferent terminals from nerves to extensor and flexor muscles[3]. Furthermore, a brief tetanic stimulation (4 stimuli at 250/sec) at Group I strength applied to the lateral gastrocnemius-soleus (LGS) nerve can induce a presynaptic inhibition of the monosynaptic reflex response evoked by stimulating the nerve to the agonist muscle MG[4,5].

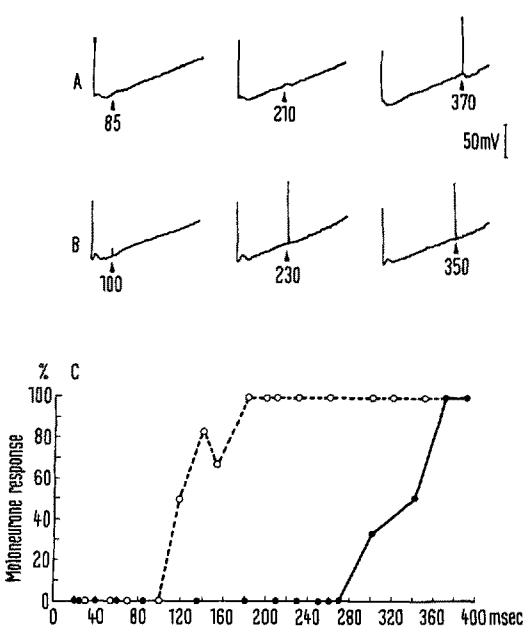
In the present experiments the mechanism involved in the inhibition of the monosynaptic reflex following a brief tetanic stimulation of Group I fibres of an agonist

extensor nerve were reinvestigated by testing the excitability of single motoneurones.

Material and methods. The experiments were performed in 9 cats, spinalized at T_{12}–L_1 level under Nembutal (35 mg/kg i.p.) anaesthesia, curarized and deefferented from L_5 to S_2; the nerves to MG and LGS muscles were isolated and cut distally. A glass micropipette, filled with 3 *M* KCl and having a resistance of 5–8 MOhms, was used for intracellular recording from alpha motoneurones, identified by antidromic stimulation.

When motoneurones were found to be monosynaptically excited by MG stimulation, the synaptic excitability was tested following brief conditioning trains (4 stimuli at 250/sec at Group I strength) applied to LGS nerve. Single test stimuli were given to MG nerve at intervals from 0–500 msec from the beginning of the conditioning stimulation. For each value of interval 6 excitability tests were repeated at intervals of 3 sec. The strength of the test stimulus was the lowest necessary to evoke, when unconditioned, a reflex discharge with a 1:1 response – stimulus ratio. With the same conditioning procedure, brief pulses of current were then given intracellularly[6], at just suprathreshold intensity, in order to test the postsynaptic membrane excitability according to FRANK and FUORTES[7].

Results. Brief tetanic stimulation of LGS nerve after a latency of about 15 msec induces usually a complete inhibition of the monosynaptic discharge of single MG motoneurones. The inhibition lasts up to 280 msec. A partial inhibition can be observed for the next 100–150 msec. A sample graph is reported in Figure C. The same electrical stimulation of LGS nerve also gives a depression of the direct response to the intracellular stimulus. This direct response is completely abolished up to 100 msec from the beginning of the conditioning stimulation and restored during the next 50–100 msec. The depression is related, as expected, with some degree of after-hyperpolarization. Similar results were obtained in LGS motoneurones following conditioning stimulation of the MG nerve. Not all the motoneurones, however, showed inhibition when tested with synaptic excitation.



Excitability tests performed in MG motoneurones following conditioning stimulation of LGS nerve. In (A) and (B) examples of intracellular recordings are reported. First spike of each record follows the LGS stimulation. At the arrows, test stimuli were applied to the MG nerve (A) and to the same motoneurone (B) following the beginning of the conditioning stimulation with the interval indicated in msec. Spikes are retouched. In the graph (C) the % of the motoneurone response (100% corresponding to a 1:1 response – stimulus ratio) to MG nerve (●–●) and direct motoneurone stimulation (o–o) are plotted against time from the beginning of the conditioning stimulation.

Discussion and conclusions. The results here reported give further evidence that the monosynaptic reflex response of single motoneurones evoked by stimulating an extensor nerve is generally inhibited for a time ranging from 150–400 msec by afferent volleys from agonist nerves. The intracellular test stimuli indicate that both postsynaptic and presynaptic inhibition cause this reflex depression. Since the excitability of postsynaptic membrane was found depressed during the first 50–200 msec in different motoneurones, after-hyperpolarization and postsynaptic inhibitory mechanisms by Renshaw cells and by Golgi tendon organs (see reference in [8]) seem to be involved. A presynaptic inhibitory mechanism, however, accounts for the subsequent inhibition lasting up to 200–400 msec, since the excitability of the postsynaptic membrane is back to normal and no hyperpolarization is found[7] after a time not longer than 200 msec.

In agreement with previous results[4,5], obtained in the same experimental condition and in which the inhibition of a monosynaptic reflex response recorded from a motoneurone pool was correlated with the primary afferent depolarization, these findings show that volleys from an extensor nerve cause presynaptic inhibition of Ia afferent terminals of agonist nerves. However, a fraction of Ia endings belonging to an extensor nerve could not be inhibited by afferent activity from an agonist nerve as no reflex inhibition was observed in some motoneurones[9].

Riassunto. La stimolazione elettrica del nervo LGS induce l'inibizione della scarcia riflessa del singolo motoneurone evocata dalla stimolazione del nervo MG. Il test di eccitabilità intracellulare dimostra che l'inibizione è provocata da meccanismi sia pre- che postsinaptici.

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