When the present results on methionine transfer are compared with previous estimations of methionine, free in the intestinal mucosa and in the extracted protein[2], it can be seen that all 3 levels increase by about the same proportion for the same shift in acclimatization temperature, implying that the ability of the mucosal cell to concentrate methionine determines its own incorporation into protein. Methionine is known to play a critical part in the aggregation of polysomes[6] and in the initiation of peptide synthesis[7] and it may act in the goldfish intestinal mucosa, in opposition to valine, as a pacemaker for other processes involved in the metabolic regulation of cells exposed to different temperatures. To reach this conclusion it has been assumed that the amounts of methionine and valine reaching the outside of the mucosal cell are always sufficiently constant to be ignored as a possible factor influencing intracellular events. This situation applies in vitro but may not be true in the intact fish where the amount of food consumed varies with the environmental temperature of the fish[8].

Zusammenfassung. Es konnte gezeigt werden, dass der Methionintransport im in-vitro-Dünndarmpräparat des Goldfischs temperaturabhängig ist: 1. Temperatur, bei welcher der Fisch akklimatisiert wurde, 2. Temperatur, bei welcher der Darm inkubiert wurde. Die Endkonzentration von Methionin auf der Serosaseite hängt ebenfalls von der Inkubationstemperatur, nicht aber von der Akklimatisationstemperatur ab. Der Flüssigkeitstransport, gemessen bei 30 °C, war um das Fünffache geringer, sobald die Akklimatisationstemperatur von 8 auf 30 °C stieg.

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Studies on Chemotaxis. VII. Cytotaxins in Rabbit Serum

Using Boyden's technique for measuring chemotaxis, the action of substances found to be chemotactic in vivo can be analysed in vitro[1]. It was shown that antigen-antibody complexes are not chemotactic per se, but exert their chemotactic effect by formation of chemotactic mediator(s) (cytotaxins)[2] in the serum. Similarly the chemotactic effect of other agents such as endotoxins, bacteria, tuberculin, glycogen or heat aggregated γ-globulins is mediated by cytotaxins formed in fresh serum[2]. The cytotaxins generated on incubation with antigen-antibody complexes are specific for polymorphs[3], and appear to differ from cytotaxins from other sources[4]. Evidence has been presented that these polymorph cytotaxins are identical with a complex consisting of the complement components C'5, 6, and 7[5]. On the other hand, it has been found that fixation of hemolytic complement and formation of cytotaxins may occur independently[6]. In the present study, further data on the properties of serum cytotaxins are reported which show that these findings are not mutually exclusive.

The thermostability of polymorph cytotaxin(s) has been tested by incubating aggregated bovine γ-globulin treated rabbit serum (aBGG) at various temperatures for 30 min. The chemotactic activity after heating was then calculated from a standard curve established in the same experiment with several concentrations of chemotactic serum heated to 56 °C. The curve given in Figure 1 is derived from the mean values of 3 experiments. The data show a pronounced loss of activity between 65 and 80 °C. A low but definite activity can still be observed at higher temperatures up to 100 °C. Whether this remaining activity is due to partial inactivation of a single cytotaxin, or to the presence of another cytotaxin differing in its thermostability, needs further investigation.

Evidence for the existence of more than one polymorph cytotaxin in rabbit serum has indeed been obtained in the following experiments. aBGG or antigen-antibody treated rabbit serum was separated on a Sephadex G-200 column using a 0.1 *M Tris*-HCl/1.0 *M* NaCl buffer pH 8.0.

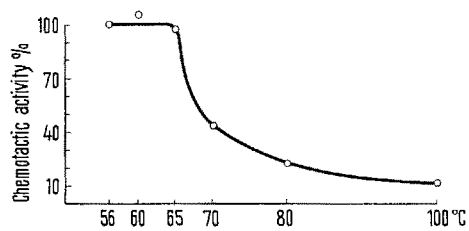

Fig. 1. Effect of heating on polymorph cytotaxins in rabbit serum.

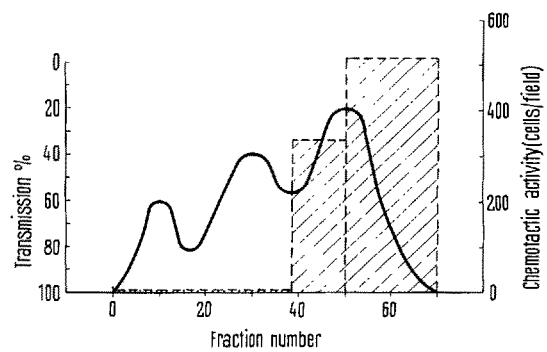

Fig. 2. Chemotactic activity of serum fractions eluted from Sephadex G-200 (eluant: 0.1 *M Tris*-HCl + 1 *M* NaCl) on polymorphonuclear leucocytes.

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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].

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

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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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