When sucrose was replaced by KCl (0.15 *M*), only 35% of the esterase activity found in the granules was released either in the cold or at room temperature, but only 10% of the activity of untreated control could be recovered from the granules.

The combined use of lauryl alcohol and glutaraldehyde protected granules against lysis in the cold but not against mechanical damage. Millipore filtration broke approximately the same percent of particles in the treated and in the control groups; only 3% to 10% was retained on filters of a pore size of 5 μm or 1.2 μm.

After 4 h of discontinous density gradient centrifugation the major portions of renin, kallikrein and esterase activities were concentrated between 1.76 *M* and 1.80 *M* sucrose solutions showing a maximum at 1.78 *M*. This distribution pattern of the enzymes was very similar to that obtained previously in a continuous sucrose density gradient[7].

Rate zonal centrifugation, however, yielded different results. After 40 min of centrifugation at 8,000 *g*, 3 distinct zones of activities were found: in the 1.1 *M*, between the 1.2 and 1.3 *M* and in the 1.5 *M* sucrose layers. The biggest difference in the distribution of the kallikrein and renin containing granules was found when their relative concentrations in the top and bottom layers were compared. While the top layers (fractions 1+2) contained 55% (52–58) of the renin and 21% (18–24) of kallikrein activity, the lower portions (1.5 *M* sucrose) had 45% (42–48) of renin and 79% (76–82) of kallikrein after rate zonal centrifugation.

Discussion. Of the enzymic activities assayed, the release of angiotensin by renin and the release of kinin by kallikrein are specific functions, while BAEe can be cleaved by kallikrein and by other enzymes as well[2]. Although most of the esterase activity of the granules from rat gland was due to kallikrein[3], other hydrolases contributed to the cleavage of BAEe in the mouse tissue[7]. Thus, we measured the esterase activity of kallikrein to detect the presence of the enzyme, while bioassay of the kinin released by the enzyme from plasma kininogen was used for the more specific determination.

We reported the first separation of kallikrein and renin containing particles intact enough for electronmicroscopic investigations[3,7]. Electron micrographs of granules obtained from the homogenized submaxillary gland of the mouse revealed a heterogenous population[7]. Some of the granules were large and spherical and resembled the kallikrein containing granules of rat submaxillary gland[3] or the zymogen granules of the pancreas[9]. Others were smaller and more amorphous, looking similar to the granules of the juxtaglomerular apparatus of the kidney[10]. The results of rate zonal centrifugation experiments reported here were taken as an indication that kallikrein granules had a bigger mean size than the renin storing granules, since a significant proportion of them sedimented in 40 min, while the relative concentration of renin granules in the more concentrated sucrose layers did not increase. Although this is not decisive evidence, it certainly supports the assumption that the large spherical granules are the ones which contain kallikrein. Rate zonal centrifugation separates the granules primarily according to their sedimentation rate, which depends predominantly on the particle size[11].

Isopycnic gradient centrifugation, which separates particles according to their density, showed a parallel distribution of esterase, kallikrein and renin activities. These results are in agreement with the electron microscopic observations that an equally dense population of granules were isolated from the submaxillary gland of the male white mouse[7].

The membranes of the granules were stabilized against lysis in the cold with lauryl alcohol and glutaraldehyde. Lauryl alcohol protected the membrane against rupture in the cold possibly by combining with a lipid component[9], while glutaraldehyde cross-linked proteins by coupling to amino groups[12]. This combined treatment, however, did not block mechanical damage to the particles during filtration.

Zusammenfassung. Subzelluläre Partikel, die Renin und Kallikrein enthalten, wurden durch Differentialzentrifugation aus homogenisierter Submaxillardrüse der männlichen weissen Maus angereichert. Durch Zonenzentrifugation wurde die relative Renin-Konzentration in der oberen Schicht des Rohrzucker-Dichtegradienten erhöht, während sich Kallikrein in der unteren Schicht anhäufte. Vorbehandlung der Partikel (granules) mit Glutaraldehyd und Lauryl-Alkohol stabilisierte sie gegen Lyse bei niedrigen Temperaturen.

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Effect of Sodium Aurothiomalate 'Myocrisin' on DNA Synthesis in Phytohaemagglutinin-Stimulated Cultures of Sheep Lymphocytes

It has been shown that the antirheumatoid drugs chloroquine and prednisolone inhibit the stimulatory effect of phytohaemagglutinin (PHA) on lymphocytes in vitro[1,2]. The purpose of the present investigation was to determine the influence of another antirheumatoid drug, sodium aurothiomalate 'Myocrisin'[3], on DNA synthesis in lymphocytes cultured in the presence of PHA.

Intestinal lymph from Dorset horn wethers approximately 18 months old was obtained by cannulating the intestinal lymphatic duct distal to the hepatic duct, using the technique described by Lascelles and Morris[4]. The lymph was collected through a polyvinyl catheter draining into a sterile bottle containing preservative-free heparin (Evans Medical, Liverpool, England). During periods when

Table I. H^3-Thymidine-labelled cells in lymphocyte cultures containing PHA and Myocrisin

Additive	Culture (h)						
	2	24	48	72	96	120	144
None	6.9 ± 0.4	0	0	0	0	0	0
PHA	7.4 ± 1.3	3.0 ± 0.7	4.4 ± 0.4	37.1 ± 3.0	35.3 ± 2.1	33.4 ± 4.3	24.6 ± 1.6
PHA and Myocrisin	7.0 ± 0.8	2.3 ± 0.8	1.4 ± 0.1	3.9 ± 1.4	17.0 ± 1.7	28.5 ± 2.2	18.9 ± 1.1
Myocrisin	6.8 ± 1.0	0	0	0	0	0	0

Table II. Dose response relationships of Myocrisin and the numbers of H^3-thymidine-labelled cells in 72 h PHA-stimulated cultures

Myocrisin (μg/ml)	Labelled cells/500 cells	Viable cells (%)
0	28.8 ± 1.6	93
5	12.8 ± 1.0	95
10	8.6 ± 0.7	98
20	8.9 ± 1.3	91
30	7.1 ± 0.4	91
50	2.3 ± 0.3	95
100	0.8 ± 0.1	91

no collection was made the intestinal lymph was returned to the circulation through another catheter inserted into the caudal vena cava. After centrifugation of lymph at 360 *g* the lymphoid cells obtained were washed in ice-cold balanced salt solution and resuspended in Medium 199 containing Polymyxin B (2 U/ml), Neomycin (1 U/ml) and 20% autologous serum. Aliquots of the cell suspension (3 ml containing 4×10^6 cells/ml) were incubated in screw cap glass culture tubes at 37 °C in a humidified incubator with 5% CO_2 in air as the gas phase for periods ranging from 2 to 144 h. In all experiments cultures were set up in quadruplicate and the influence of PHA and Myocrisin, either alone or in combination, was examined. PHA (MR10, Burroughs Wellcome & Co.) was added in a concentration of 0.02 ml/ml culture medium and Myocrisin (May and Baker, Dagenham, England), diluted with double-distilled water, was added to the cultures to give a concentration of up to 100 μg/ml of culture medium. The proportion of viable cells in each culture was estimated by removing 0.5 ml of culture fluid 2 h before termination of each culture and incubating the fluid with 0.1 ml of an 0.4% solution of trypan blue for 15 min at room temperature. Both the total number of cells and the number of stained cells present in each culture were then counted in a haemocytometer.

To study DNA synthesis, the cultures were given a pulse of 2 μc/ml H^3-thymidine (specific activity 2000 mC/m*M*) 1 h before the cultures were terminated. After washing once in ice-cold balanced salt solution, the cells were resuspended in a small volume of homologous serum and thick smears prepared on acid-cleaned glass slides were air-dried for 15 min and fixed in absolute methanol for 10 min. Slides for autoradiography were coated with Ilford K2 emulsion at 45 °C (1 part emulsion and 1 part distilled water) and stored at 4 °C for 21 days in light-tight boxes containing dessicant. Slides were exposed to a developer (Ilford 1D-19) and, after fixation in hypo, were rinsed and stained by the May-Grunwald Giemsa method. The mean number of labelled cells in each individual culture was determined from counts of 500 cells made on duplicate smears. The mean number of labelled cells ± S.E.M. was then determined for each group of 4 cultures (Tables I and II).

DNA synthesis as assessed by the incorporation of H^3-thymidine in nuclei occurred in cultures maintained in the presence of PHA but no corresponding effect was seen in cultures maintained in the presence of Myocrisin alone. DNA synthesis reached a peak at 72 h and peristed for as long as 144 h in lymphocytes cultured in the presence of PHA alone (Table I). In culture media containing PHA the addition of Myocrisin led to a delay of approximately 24 h in the peak observed in the number of labelled cells present. The effects of different doses of Myocrisin on PHA-stimulated cultures are shown in Table II. In a series of cultures maintained for 72 h there was a pronounced reduction in the number of labelled cells in those containing 5 μg/ml of Myocrisin and a still further but smaller reduction in cultures containing higher concentrations of Myocrisin; DNA synthesis was virtually absent in cultures containing 100 μg/ml of Myocrisin. Findings shown in Table II also indicate that the viability of lymphocytes under the experimental conditions adopted is not appreciably impaired by Myocrisin.

It seems clear from the present studies that Myocrisin inhibits, but not irreversibly, DNA synthesis in lymphocytes cultured in the presence of PHA. Suggestions that the release of lysosomal enzymes is essential for DNA synthesis have been made[5,6] and the possibility thus exists that Myocrisin exerts its inhibitory effect by a stabilizing action on lysosomal membranes as has been found in the case of other antirheumatoid drugs[7].

Zusammenfassung. Das Antirheumatikum Natrium-Aurothiomalat «Myocrisin» hemmt reversibel die durch Phytohaemagglutinin angeregte Synthese von DNS in Kulturen von Lymphozyten des Schafes.

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