we used the same concentration expressed in anatomical units: 18 heads per ml, and the activity was 23.4 and 18.7 units per mg of protein respectively. When the whole homogenate was reconstitued by mixing the homogenized eyes in water with the homogenate of the rest of the heads, the activity became almost the same as that measured in the extract of whole heads. The eye extract in 15 mM DOC was shown to be a good inhibitor of insect AChEs (table). Enzymes from the heads of housefly *(Musca domestica)*, *Rhodnius prolixus*, cockroach *(Periplaneta americana)* and *T. infestans*, free of eyes, were inhibited by the colored homogenate of *T. infestans* eyes. In contrast no inhibitory activity was observed against mammalian cholinesterase as shown in the table.

We obtained an indirect proof of the localization of the inhibitor during the *T. infestans* embryogenesis. In previous work in our laboratory an embryonic development of 15 days until hatching was found[11]. The appearance of the compound eyes in the embryo was observed on the 7th day of development.

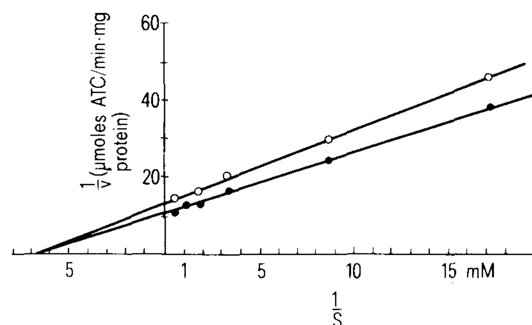
Taking into account the presence of the eyes, a boiled homogenate of 7-day-old eggs of *T. infestans* was assayed as an inhibitor. AChE from *T. infestans* heads free of eyes was inhibited 30% by a final concentration of 2.5 eggs per ml. In contrast, similar homogenates of 4–6-day-old eggs, in which the embryos lack visible eyes, did not show any inhibitory activity against insect AChE.

The eye extract in 15 mM DOC was subjected to electrophoresis in order to separate the ocular pigments present in the *T. infestans* homogenates. These pigments appear to be related to the AChE inhibition. We observed 2 partially overlapping pigments, violet and yellow respectively, with high electrophoretic mobility in our conditions.

The gel column was transversely sectioned into 10 pieces and each one was homogenized with a homogenate of housefly head (AChE activity: 71.5 units/mg protein) under similar conditions and incubated for 10 min. All the homogenates showed the same activity except the one containing both ocular pigments. In the latter case the hydrolysis rate of ATC was 70% inhibited.

With regard to the properties of the inhibitor, the K_m of housefly head AChE was determined with and without extract of *T. infestans* eyes. The results shown in the figure point out the noncompetitive characteristic of the inhibitor because of the similar K_m-value obtained in both cases (1.5×10^{-4} M).

Taking into account the above results, the inhibitor associated with the pigment from the *T. infestans* eyes could be defined as reversible, noncompetitive and selective against insect AChE.



Noncompetitive inhibition of housefly head AChE by homogenate from *Triatoma infestans* eyes. Enzyme activity was 71.5 units/mg protein and the incubation time 5 min. Results are expressed according to the method of Lineweaver and Burck (J. Am. chem. Soc. *56*, 658 (1934)). ● control, ○ inhibition (final concentration 3.5 eyes per ml).

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Time courses and refractoriness of enhanced vascular permeability induced by histamine, serotonin and bradykinin in synovialis of the rat

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Summary. Enhanced vascular permeability induced in synovialis of the rat by histamine and serotonin lasts 5–15 min and that induced by bradykinin less than 5 min. Synovialis of the rat becomes refractory to the permeability effects of repeated doses of each of these substances in the hour following initial application.

Histamine, serotonin and bradykinin are known to enhance the permeability of the vasculature of synovialis in several mammalian species including the rabbit[3], monkey[4], rat[5] and dog[6]. In synovialis of the rat, serotonin is more potent than bradykinin, which is in turn more potent than histamine[5].

This paper reports an investigation of the duration of the permeability effects of histamine, serotonin and bradykinin on synovialis of the rat and of refractoriness of synovial vessels to repeated applications of these substances.

Materials and Methods. Albino rats of both sexes (b. wt 250–350 g) were used throughout and were lightly anaesthetized with ether for all injections.

Increased vascular permeability of synovialis of the stifle joints of the rats was detected by the use of i.v. injections of colloidal carbon as previously described[5]. After i.v. injection, carbon is removed from the circulation by the reticuloendothelial system within 1 h, but also collects in the walls of abnormally permeable blood vessels[7]. Each animal received colloidal carbon (Gunther Wagner, CII/1431a,

Pelikan, Hanover) 0.1 ml/100 g b. wt i.v., and was killed 75 min later. The membrane of each stifle was then exposed and examined with a dissecting microscope. The amount of carbon deposited in the walls of the synovial vessels (referred to as the 'labelling' of the vessels) of each joint, served as an index of increased vascular permeability and was scored on an arbitrary scale 0-5.

Solutions of histamine acid phosphate, serotonin creatinine sulphate and bradykinin which evoke marked permeability responses in rat synovialis (360 µg histamine base/ml, 5 µg serotonin base/ml and 10 µg bradykinin/ml)[5] were prepared in Tyrode solution at pH 7.3. The volume of each intra-articular injection was 0.05 ml, providing individual doses of 18 µg histamine base, 0.25 µg serotonin base and 0.5 µg bradykinin respectively per joint for each injection.

The duration of the response of synovial membrane to each substance was studied by injecting the appropriate solution into the right and left stifle joints of 3 groups of 5 rats. Each group of animals received i.v. colloidal carbon either just prior (referred to as '0') or after 5 or 15 min of injection of the test substance into the joints. 3 additional groups of 5 rats, the joints of which were injected with Tyrode solution alone, were given i.v. colloidal carbon after similar intervals and served as controls.

Refractoriness of the permeability response of synovial vessels to histamine, serotonin and bradykinin was tested by injecting joints of animals twice with the appropriate solution. The injections were separated by various intervals of time and the permeability effect of each of the 2nd injections was estimated by administering animals with i.v. colloidal carbon once only shortly before this 2nd injection. The time intervals between intra-articular injections were 1. slightly longer than the duration of the permeability effect of a single injection of the respective substance (see above), 2. 1 h, 3. 3 h and 4. 6 h. For each interval and for each substance, the right and left stifle joints of 5 rats were

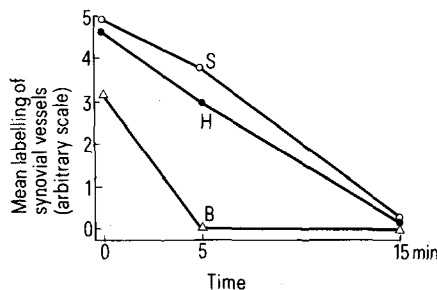

Fig. 1. Time-courses of increased vascular permeability evoked by histamine (H), serotonin (S) and bradykinin (B) in synovialis of the rat.

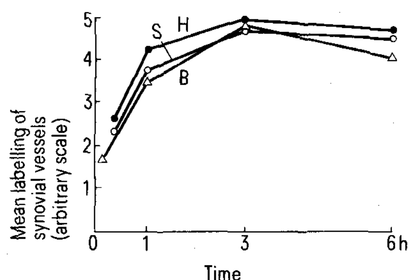

Fig. 2. Vascular permeability evoked in synovialis by the 2nd of 2 injections of histamine (H), serotonin (S) and bradykinin (B), the intervals between repeated injections ranging from several minutes to 6 h. Refractoriness of the vessels to the substances occurred only with the shortest intervals between injections.

used, while the joints of further groups of 5 rats were injected twice with Tyrode solution after the corresponding intervals to serve as controls.

Results and discussion. The permeability response evoked by each substance was brief (figure 1). For histamine, labelling of synovial vessels of animals receiving colloidal carbon immediately before injection of the joints was intense, with a mean score of 4.7. However, labelling in animals receiving colloidal carbon 5 min after intra-articular injection was of moderate intensity (mean score 3). In the group of animals receiving colloidal carbon 15 min after injection of the joints, vascular labelling was found in only 1 of 10 joints, and was of minimal intensity, with a score of 1. For serotonin, the duration of the response of the synovial vessels was similar to that evoked by histamine. Marked labelling (mean score 5.0) was present in joints of animals receiving colloidal carbon shortly before injection of the joints while moderate labelling (mean score 3.7) was present in joints of animals receiving colloidal carbon 5 min after intra-articular injection. The labelling occurring in joints of animals receiving colloidal carbon after 15 min was minimal (mean score 0.2). For bradykinin, however, the duration of the permeability response was even shorter than those for histamine and serotonin. Labelling of synovial vessels, which was moderate (mean score 3.2) in animals receiving colloidal carbon shortly before injection of the joints, was minimal in animals receiving colloidal carbon after either 5 or 15 min of such injections. The means of the scores of the labelling after these intervals were 0.2 and 0.0, respectively.

Refractoriness of vessels of synovialis to 2nd intraarticular injections of histamine, serotonin and bradykinin varied according to the intervals between such injections, although the pattern of such responses was similar for each substance (figure 2). For histamine, a 2nd injection given 20 min after the first evoked only a moderate permeability response, the mean score of labelling in test joints being 2.7. However, 2nd injections given 1, 3 or 6 h after the first evoked marked permeability responses which approximated those evoked by a single injection of the same dose of histamine. The means of the labelling scores for these groups were 4.3, 5.0 and 4.8 respectively.

Similarly for serotonin, the intensity of vascular labelling evoked by a 2nd injection given 20 min after the first was moderate (mean score 2.3), while the responses evoked by 2nd injections given 1, 3 and 6 h after the first approximated that induced by a similar single injection of serotonin (the mean scores being 3.8, 4.8 and 4.6 respectively). Also for bradykinin, the intensity of labelling evoked by 2nd injections given 6 min after the first was mild (mean score 1.7) and those induced by 2nd injections after intervals of 1, 3 and 6 h were similar to those induced by a single injection (mean scores 3.6, 4.9 and 4.1 respectively).

The duration of enhanced vascular permeability evoked by histamine and serotonin (5-15 min) and bradykinin (less than 10 min) in synovialis of the rat correspond to the duration of their effects in the skin of this animal[8,9]. However, the refractoriness of synovial vessels to all 3 substances in the 1st h after a previous injection found in the present study is unlike the results of similar studies in rat's skin. Oyvin et al.[9] found that rat dermal blood vessels develop refractoriness to histamine only after 30 min of a 1st injection of this substance, and demonstrated no refractoriness of these vessels to bradykinin after any interval. Refractoriness of dermal blood vessels to histamine but not bradykinin has also been demonstrated for the guinea pig[10] and man[11].

The present results indicate that in synovialis, the duration of enhanced vascular permeability evoked by histamine, serotonin and bradykinin is brief and that a short period of

refractoriness occurs following application of each substance.

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Ultrastructural studies of Morris hepatoma cells reversely transformed by ginsenosides

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Summary. Ginsenosides, which were extracted from Panax ginseng, C.A. Meyer, induced well the development of subcellular organelles in cultured Morris hepatoma cells (MH_1C_1).

The basic action of an extract from the root of Panax ginseng, C.A. Meyer, has been reported to be the stimulation of various metabolic reactions of liver cells in vivo[2-4]. Recently, Odashima et al.[5] have shown that ginsenosides induce a reverse transformation of Morris hepatoma cells (MH_1C_1) as follows. The ability of these cells to grow in 0.33% soft agar suspension culture, which is one of the assays for neoplastic transformation of cells, was reduced to one-fourth of that of the control cells. In addition, a remarkable increase of L-^{3}H-ornithine in arginine deficient medium, an increase in the activity of succinate-cytochrome c reductase and a decrease in the activity of 5′-nucleotidase were also observed. These results suggest that ginsenosides might induce the reverse transformation of MH_1C_1 cells. The present paper describes ultrastructural features of these cells to provide further documentation of the structure and function of these cells.

Materials and methods. Extraction and purification of ginsenosides. Ginsenosides were isolated from the roots of Panax ginseng, C.A. Meyer, as described in a previous report[5]. Cells and growth media. Morris hepatoma cells (MH_1C_1) were grown in Leibovitz L-15 medium supplemented with 10% fetal serum, 50 units/ml of penicillin and 50 µg/ml of streptomycin. The effects of ginsenosides on MH_1C_1 cells were examined using a culture medium containing 100 µg/ml of ginsenosides. The details of our procedures for observing the behaviour of ginsenosides have been previously reported[6-8]. Light and electron microscopy. Cultured MH_1C_1 cells were examined under normal and inverted phase contrast microscopes and under the electron microscope according to the following method. Cultured cells were fixed in situ with 2% glutaraldehyde and post-fixed in 1% osmium tetroxide. After dehydration in graded ethanol solutions, the cultures were covered with Epoxy resin. Capsules filled with epon were inverted over the cultures and the epon polymerized. Sections were stained with uranyl acetate and examined at 100 kV with a Hitachi HS-9 electron microscope.

Results and discussion. Morris hepatoma cells (MH_1C_1) have an irregular form and are relatively small in size ($18.95 \pm 0.78 \times 11.33 \pm 0.58$ µm). The cells have a large nucleus with prominent nucleoli, and scanty cytoplasm as shown in figure 1. The Morris hepatoma cells cultured in the medium containing 100 µg/ml of ginsenosides for 24 subcultures (about 170 days) are much larger ($32.68 \pm 0.8 \times 21.53 \pm 0.65$ µm) than the control Morris hepatoma cells and have abundant cytoplasm containing numerous small particles. They form a typical epitherial pattern and the appearance of them is very similar to that of normal cultured liver cells (figure 2)[5,7]. This process was named 'reverse transformation or redifferentiation' of Morris hepatoma cells, since it appeared to reverse the formation of many of the characteristics assumed by cells treated with malignant transforming agents[5].

Electron microscopically, Morris hepatoma cells are characterized by small mitochondria and a decreased rough endoplasmic reticulum. Mitochondria are sparse and pleomorphic, averaging 0.2–2.0 µm in diameter as shown in figure 3. Rough endoplasmic reticulum is distributed randomly and has never been seen to form large stacks.

In contrast with this, the subcellular organelles of Morris hepatoma cells cultured in medium containing ginsenosides are well developed and their distribution is also well organized. Mitochondria increase remarkably in number and size (0.5 ± 0.2 µm in diameter and 1.3 ± 0.1 µm in length). Most of these mitochondria with a matrix of normal density are encircled by single cisternae of rough endoplasmic reticulum (figure 4). This observation seems to be closely related to the results, reported in a previous paper[5], that the activities of succinate-cytochrome c reductase and ornithine uptake in arginine deficient medium were significantly increased in reversely transformed Morris hepatoma cells. Golgi complexes are also prominent and consist of enlarged cisternae of smooth membrane (figure 5). Some of these Golgi complexes seemed to be polarized towards the structures resembling bile canaliculi which developed well in comparison with control Morris hepatoma cells.

This extensive development and reorganization of subcellular organelles is consistent with our unpublished result[9] that ginsenosides stimulate protein synthesis, particularly production of albumin and α-globulin, in MH_1C_1 cells.

Another structural characteristic of the cells reversely transformed by ginsenosides is that desmosomes are well developed and tonofilaments are seen on the electron dense desmosome plate (figure 6). This result is one of the most important pieces of evidence, since the cells in tumors arising in vivo fail to develope desmosomes[10] and even in normal cultured liver cells the establishment of desmosomes is rarely encountered[11].

These morphological observations on the reversely transformed cells compare well with those obtained by biochem-