gende Familien von Bodenvögeln zählen hierzu[3,8]: Phasianiden, Numididen, Ralliden, Heliornithiden, Pteroclidden, Phalaropodiden, Jacaniden, Arenariiden, die meisten Charadriiden, ferner viele Otiden, Anatiden. Ebenso sehen auch diejenigen Sterniden aus, die keine Fische fressen (zum Beispiel *Chlidonias leucoptera*). Bei den Podicipiden und Colymbiden ist der Körper nur soweit mitschattiert, als er aus dem Wasser herausragt, der Bauch bleibt weiss (siehe oben).

Die Korrelation der Mitschattierung mit dem Bodenleben zeigen Vertreter aus solchen Vogelgruppen besonders eindrucksvoll, die zum grössten Teil nicht auf dem Boden leben: Der Sekretär unter den Greifvögeln, viele Ammern unter den Emberiziden, von den Timaliiden Südafrikas eine auf Felsen lebende Art, ebenso unter den Turdiden viele Felsen oder Ödland bewohnende Arten. Von 59 Sylviidenarten Südafrikas lebt nur eine einzige auf Felsen und diese ist mitschattiert. Die Ploceidenarten Südafrikas lassen sich wie folgt aufteilen:

	Bauch schwarz	Bauch weiss
Bodentier	24	8
Baumtier	2	18

dazu 14 Arten, die keiner der vier Gruppen zugeordnet werden können.

Neben der Mitschattierung spielen jedoch noch andere Faktoren eine Rolle, so dass das Bild nicht immer so klar ist. Die acht weissbäuchigen Weber können sich entweder keinen dunklen Bauch leisten, oder sie sind phylogenetisch junge Bodentiere. Dasselbe gilt für die Lerchen. Von 26 südafrikanischen Arten sind nur 3 mitschattiert, alle anderen kryptisch gefärbt.

Summary. Contrast and concealment colour patterns take the form of spotting in visually articulated surroundings. In visually more uniform surroundings, the same effects are achieved by means of shading. The present paper brings some new examples of 'countershading', and explains the phenomenon of 'shadow accent' (the reverse of countershading: emphasis of shadow areas by pigmentation, increasing the contrast between the animal and its environment).

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[8] R. PETERSON, *A Field Guide to the Birds, A Field Guide to the Western Birds* (The Riverside Press, Cambridge, Mass., 1960), p. 230 und 240. – R. PETERSON, G. MOUNTFORT und P. A. D. HOLLOM, *A Field Guide to the Birds of Britain and Europe* (Collins, London 1954), p. 318. – A. ROBERTS, *The Birds of South Africa* (Cape Times Ltd., Cape Town 1958), p. 512.

STUDIORUM PROGRESSUS

Mast Cells and Anaphylaxis

Introduction. It is now well established that mast cells of certain species, e.g. guinea-pig[1–3], mouse[4–6], rat[7,8], hamster[9], dog[10–12], rabbit[3], and man[13], are damaged during anaphylaxis and in anaphylactoid reactions. The morphological changes observed comprise swelling and vacuolization of the cells and the extrusion of their characteristic granules into the adjacent connective tissue[14–16]. At the same time, the substances contained in the granules in relatively large quantities, particularly histamine and heparin—and in rats and mice serotonin additionally[15]—are released. Under certain conditions, such as in anaphylaxis[17–19], as well as by the action of the chemical histamine-liberator compound 48/80[20,21], a slow reacting substance (SRS) has also been detected which causes the smooth muscle slowly to contract. Its formation is said to run parallel to the release of histamine *in vivo*[17,18] and it has recently been demonstrated in work with isolated mast cells[21]. It may thus be assumed that it, too, stems from the mast cells in the course of their disruption. So far, SRS has defied identification, although there are reasons for believing that it consists mainly of unsaturated fatty acids[22]. However, ARCHER[23] believes that SRS is 5-hydroxytryptamine. In view of the important role played by the mast cells in the organism, the question thus arises: what is the mechanism of mast-cell damage during anaphylaxis? Is it due to the antigen-antibody-reaction which takes place at the level of the mast cell, or to a disruption effect of an active material formed elsewhere as a result of the union of antigen and antibody[24,25]? Improvements in the technique of the method originally described by PADAWER and GORDON[26] for the isolation of mast cells from rat peritoneal fluid, such as replacement of hypertonic sucrose by 50% Dubos bovine albumin[27,1] or a high-molecular polysaccharide ('Ficoll')[28,29] as media for differential centrifugation have now enabled the reactions of isolated mast cells to be studied *in vitro*.

[1] I. MOTA, J. Physiol. (Lond.) *147*, 425 (1959).
[2] I. MOTA and I. VUGMAN, Nature (Lond.) *177*, 427 (1956).
[3] E. G. STUART, Anat. Rec. *112*, 394 (1952).
[4] P. B. CARTER, R. D. HIGGINBOTHAM, and TH. F. DOUGHERTY, J. Immunol. *79*, 259 (1957).
[5] R. D. HIGGINBOTHAM, in Fourth Conference on Polysaccharides in Biology (Josiah Macy, Jr., Foundation, 1959), p. 171.
[6] S. TOKUDA and R. S. WEISER, J. Immunol. *86*, 292 (1961).
[7] R. KELLER, Int. Arch. Allergy *11*, 328 (1957).
[8] I. MOTA, Brit. J. Pharmacol. *12*, 452 (1957).
[9] O. WEGELIUS, G. HJELMMAN, and C. WASASTJERNA, Acta path. microbiol. scand. *36*, 309 (1955).
[10] L. B. JAQUES and T. WATERS. J. Physiol. (Lond.) *99*, 454 (1941).
[11] A. E. SCROGGIE and L. B. JAQUES, J. Immunol. *62*, 103 (1949).
[12] O. WILANDER, Skand. Arch. Physiol. *81*, Suppl. 15 (1938).
[13] G. SALVATO, Int. Arch. Allergy *18*, 348 (1961).
[14] J. F. RILEY, J. Path. Bact. *65*, 471 (1953).
[15] J. F. RILEY, *The Mast Cells* (Livingstone, Edinburgh 1959).
[16] J. F. RILEY and G. B. WEST, *Heffter's Handbuch der Pharmakologie* (Springer Verlag), in Vorbereitung, engl.
[17] W. E. BROCKLEHURST, J. Physiol. (Lond.) *151*, 416 (1960).
[18] N. CHAKRAVARTY, Acta physiol. scand. *48*, 167 (1960).
[19] N. CHAKRAVARTY and B. UVNÄS, Acta physiol. scand. *48*, 302 (1960).
[20] N. CHAKRAVARTY, B. HÖGBERG, and B. UVNÄS, Acta physiol. scand. *45*, 255 (1959).
[21] B. UVNÄS and I.-L. THON, Exp. Cell Res. *23*, 45 (1961).
[22] R. M. SCHÜTZ and W. VOGT, Arch. exp. Path. Pharmak. *240*, 504 (1961).
[23] G. T. ARCHER, Nature (Lond.) *190*, 350 (1961).
[24] H. GIERTZ, F. HAHN, W. OPFERKUCH, and W. SCHMUTZLER, Arch exp. Path. Pharmak. *242*, 42 (1961).
[25] H. GIERTZ, F. HAHN, I. JURNA, and W. SCHMUTZLER, Arch. exp. Path. Pharmak. *242*, 65 (1961).
[26] J. PADAWER and A. S. GORDON, Proc. Soc. exp. Biol. Med. *88*, 29 (1955).
[27] R. KELLER, Pathol. Microbiol. *24*, 932 (1961).
[28] R. KELLER and I. BEEGER, Med. exp. *4*, 51 (1961).
[29] B. UVNÄS and I. L. THON, Exp. Cell Res. *18*, 512 (1959).

Morphological changes of mast cells in anaphylaxis. A number of earlier investigations had shown that the mast cells swell and become degranulated during anaphylactic shock in the intact dog[10–12], guinea-pig[2,3], rabbit[3], mouse[4,5], and rat[7,8]. A similar reaction of mast cells *in vivo* was observed upon administration of rabbit anti-hamster serum to hamsters[9], rabbit anti-rat connective tissue serum[7] or anti-rat mast cell serum to rats[30], but not in anaphylaxis of the mouse produced by soluble antigen-antibody complexes[6]. Quantitative studies on mast cells, which were aimed mainly at the investigation of the mechanism of the anaphylactic reaction, have in recent years been performed principally on isolated mesenteries of the guinea-pig[1,31,32] and the rat[31,33–36] and on chopped lung tissue[37,38]. In addition, BORÉUS[39] has studied, *in vivo*, the qualitative and quantitative changes of the mast cells in the nasal mucosa of sensitized guinea-pigs following intravenous, intra-arterial or topical administration of antigen. Fundamental observations[32] on the mast cells of guinea-pig mesenteries have shown that reversed passive anaphylaxis and mast cell damage occurred when the antigen was a suitable γ-globulin, but not if it was an albumin. Antiserum against homologous γ-globulin caused typical anaphylaxis and mast-cell degranulation, whereas antiserum against Forssman antigen caused capillary damage without mast-cell change; antiserum against homologous albumin was ineffective[32].

Observation under the phase-contrast microscope revealed that living mast cells isolated from actively sensitized rats by the methods mentioned above undergo morphological damage upon addition of the specific antigen (horse serum[29,40,41], egg-white[41] crystallized ovalbumin[28], human γ-globulin[28]) in a similar manner as by a chemical histamine-liberator, such as compound 48/80[21,29]; granules are disrupted, and swelling of the cells with concomitant extrusion of granules is observed within 30–90 sec after addition of the antigen. However, the reaction takes place only in a proportion of the cells, the number varying greatly from one animal to the other. A similar mast-cell reaction can be observed, following passive sensitization, both *in vivo* and *in vitro*[42], as well as after reversed passive sensitization[43], upon addition of the antigen or the antibody, but again not all mast cells appear to be equally susceptible. In contrast, when rabbit anti-rat serum is allowed to act on mast cells isolated from normal rats, *all* the cells display extensive morphological changes[44]. This action is entirely due to antibodies against rat globulin whereas rabbit anti-rat albumin serum produces no such effects[43,45]. It may thus be concluded that mast-cell damage occurs in anaphylaxis as the result of an antigen-antibody reaction itself. One of the two reactants is presumably reversibly fixed to the mast-cell surface and, on reacting with the second component, leads to the activation of an enzymatic process which damages the cell. MOTA[46] has recently shown that only lytic antibodies are effective in releasing histamine from mast cells of the rat.

Histamine release from mast cells in anaphylaxis. Evidence that mast cells themselves contain relatively large quantities of histamine explains the parallelism, observed many years ago, between histamine release and mast-cell disruption[10] in anaphylaxis. The relationship is particularly close in the guinea-pig[1,2], less so in the rat. The release of histamine takes place, as do the morphological mast-cell changes, in a matter of seconds: it depends on the concentration of antigen[1,18,47–49], on temperature[19,27,28,34,38,40,42,44,45,48,49], pH value[19,34,48], and ionic strength[50,51], and may be retarded or suppressed by substances having enzyme-inhibitory properties (see references[1,19,27,28,34,37,42,4448,50,52–55]).

The amount of histamine released from mast cells isolated from *actively* sensitized rats upon addition of the antigen varies greatly from one animal to another (0–70%); generally it is relatively small (20–30%). The quantities of histamine released from mast cells after *passive* or reversed passive sensitization are very small indeed. On the other hand, anti-rat serum[44] and, particularly, anti-rat γ-globulin serum[45,52] are capable of liberating most of the mast-cell histamine, whereas rabbit anti-rat albumin serum as well as normal rabbit serum do not. The data obtained from the study of isolated cells show that, under certain conditions, anaphylactic mast-cell damage and histamine release can take place without the intervention of other cells or agents. However, it is still possible that substances with histamine-liberating properties, formed elsewhere, may be of some importance for histamine release from mast cells in the intact animal[56,57].

The mechanism of the anaphylactic mast-cell reaction. Experiments made in recent years, particularly on chopped lung tissue, have clarified the biochemical processes essential for anaphylaxis[37,38,51]. The results so far obtained on the anaphylactic mast-cell changes on isolated mesenteries or lungs of rats[34,39,48,54;55] and guinea-pigs[1,18,48,55] are in good agreement and will be discussed later in detail in the light of work done on isolated mast cells. Particularly interesting are the quantitative investigations of HUMPHREY and MOTA[32] on the mast cells of the guinea-pig mesentery. These workers have shown that the mast-cell response of passively sensitized guinea-pigs to antigen is well correlated with the extent of the anaphylactic shock. Multiple sensitization and subsequent contact with several different antigens caused a cumulative, but not complete, disappearance of the cells.

The anaphylactic reaction of isolated mast cells, irrespective of the way in which it is elicited, depends on *temperature*, the maximum effect being observed at 37–40°C; at temperatures below 15°C and above 45°C it

[30] D. E. SMITH and Y. S. LEWIS, J. exp. Med. *113*, 683 (1961).
[31] L. O. BORÉUS, Acta physiol. scand. *49*, 251 (1960).
[32] J. H. HUMPHREY and I. MOTA, Immunology (Lond.) *2*, 31 (1959).
[33] B. HÖGBERG and B. UVNÄS, Acta physiol. scand. *41*, 345 (1957).
[34] B. HÖGBERG and B. UVNÄS, Acta physiol. scand. *48*, 133 (1960).
[35] B. UVNÄS, Ann. N.Y. Acad. Sci. *90*, 751 (1960).
[36] B. UVNÄS, B. DIAMANT, B. HÖGBERG, and I.-L. THON, Amer J. Physiol. *199*, 575 (1960).
[37] J. L. MONGAR and H. O. SCHILD, J. Physiol. (Lond.) *135*, 301 (1957).
[38] J. L. MONGAR and H. O. SCHILD, J. Physiol. (Lond.) *135*, 320 (1957).
[39] L. O. BORÉUS, Acta physiol. scand. *48*, 431 (1960).
[40] R. KELLER, Helv. physiol. Acta *19*, C 27 (1961).
[41] I. MOTA and W. D. DA SILVA, Nature (Lond.) *186*, 245 (1960).
[42] R. KELLER and M. SCHWARZ, Schweiz. Med. Wschr. *91*, 1196 (1961).
[43] R. KELLER and M. SCHWARZ, Med. exp., *5*, 109 (1961).
[44] R. KELLER and M. SCHWARZ, Int. Arch. Allergy *19*, 203 (1961).
[45] R. KELLER, Nature (Lond.) *193*, 282 (1962).
[46] I. MOTA, Nature (Lond.) *192*, 1201 (1961).
[47] L. O. BORÉUS and N. CHAKRAVARTY, Acta physiol. scand. *48*, 315 (1960).
[48] N. CHAKRAVARTY, Acta physiol. scand. *48*, 146 (1960).
[49] R. KELLER, Helv. physiol. Acta, *19*, C 79 (1961).
[50] R. KELLER, unveröffentlichte Ergebnisse.
[51] K. F. AUSTEN and W. E. BROCKLEHURST, J. exp. Med. *113*, 521, 541 (1961); *114*, 29 (1961).
[52] K. F. AUSTEN and J. H. HUMPHREY, J. Physiol. (Lond.) *158*, 36 P (1961).
[53] O. FERNÖ, B. HÖGBERG, and B. UVNÄS, Acta pharmacol. toxicol. *17*, 18 (1960).
[54] I. MOTA, W. D. DA SILVA, and J. F. FERNANDES, Brit. J. Pharmacol. *15*, 405 (1960).
[55] I. MOTA and T. ISHII, Brit. J. Pharmacol. *15*, 82 (1960).
[56] G. T. ARCHER, Nature (Lond.) *182*, 726 (1958).
[57] N. J. GIARMAN, L. T. POTTER, and M. DAY, Exper. *16*, 492 (1960).

is completely inhibited[27,28,40,42,44,45,50]. It also requires *complement*. Thus, if the guinea-pig serum in the medium is replaced by serum decomplemented by heat (30 min 56° C) or serum obtained from zymosan pretreated guinea-pigs or if the medium contains EDTA ($10^{-4}M$), then the reaction is greatly suppressed[28]. Anaphylaxis *in vitro* can also be inhibited by a number of substances which *inhibit enzymes*, e.g. metal ions (Zn, Pb, Cu), amino-group reagents (1-fluoro-2, 4-dinitro-benzol, formaldehyde), sulfhydryl-group reagents (iodoacetic acid, maleinimides, *o*-iodosobenzoate, *p*-chloromercuribenzoate) and substances which interfere with oxidative energy-regenerating processes (cyanides, azides)[28,42,44]. These substances exert inhibitory effects on the anaphylactic mast-cell reaction both in active and reversed passive sensitization as well as on the reaction elicited by antibodies against rat serum or rat globulin. However, they have no influence on the reaction of passively sensitized mast cells[42].

The above findings thus show a dependence of the anaphylactic reaction of isolated mast cells on temperature and on the presence of complement, and that it can be prevented by many substances with enzyme-inhibiting properties. Presumably, therefore, it is enzymatic. Similar results and conclusions had been arrived at in earlier research on chopped guinea-pig lung tissue[18,19,37,38,48,51,54] and on isolated rat mesenteries[33–36,53]. This last-mentioned observation induced HÖGBERG and UVNÄS[33] to study the influence of about 30 enzymes on mast cells of isolated rat mesenteries. These authors found only one enzyme with a disruptive action on mast cells, viz. lecithinase or phosphatidase A. Its effect, as well as the disruptive action of the potent chemical histamine-liberator, compound 48/80, was inhibited by a number of substances with enzyme-inhibiting properties. These observations induced HÖGBERG and UVNÄS[33] to put forward the most attractive hypothesis that an enzyme similar to, or identical with, phosphatidase A is attached to the mast cell membrane; histamine-liberators of the type of compound 48/80 would accordingly operate by removing an inhibitor normally blocking the enzyme.

These findings, obtained from a study of rat mesenteries, have been confirmed on isolated mast cells[27]. It was shown in addition, however, that α-chymotrypsin, although requiring about ten times larger doses than phosphatidase A, also exerts mast-cell disrupting and histamine liberating effects[27]. However, it still remains to be determined to what extent such mechanisms operate *in vivo*. On the other hand, it was found that a number of substances with chymotrypsin inhibiting properties like di-isopropylfluorophosphate, *p*-nitrophenylacetate, ε-amino caproic acid, nicotinamide and the like[27,42,50,52], as well as a number of chymotrypsin substrates[49,50,52], do exert a distinct inhibitory effect on the anaphylactic reaction of isolated rat mast cells. These results are in good agreement with the findings of AUSTEN and BROCKLEHURST[51], and MOTA, DA SILVA, and FERNANDES[54] on the inhibition of the anaphylactic reaction in the guinea-pig lung. BENDITT and ARASE[58] observed that mast cells isolated from the normal rat exhibit chymotrypsin-like activity which is inhibited by DFP; probably the mast cell enzyme was activated during isolation; moreover, histochemical methods have likewise shown a high concentration of proteolytic enzymatic activity in the cells[59,60]. The above results thus justify the consideration of chymotrypsin-type proteolytic enzymes, besides phosphatidase A, in further investigations concerning the mechanism of histamine release from mast cells.

Species differences in anaphylaxis. The findings cited above refer exclusively to the mast cells in the albino rat. However, it is well known that there exist substantial differences in the anaphylactic pattern from one species to another. While the mast cells of rats[31], mice[4,5], hamsters[9,31], and men[61] display a certain similarity as regards their behaviour, those of the guinea-pig[31] behave differently. For example, the latter do not respond to the histamine-liberator, compound 48/80, which is so effective in the other species, expecially the rat[31,62]. On the other hand, mast cells of the rat respond less vigorously and less regularly than those of the guinea-pig during anaphylaxis[49,63]. Recent histochemical experiments[59] also show that the mast cell granules contain a number of enzymes capable of exercising pronounced activity and that there exist marked differences in the enzyme pattern of the cells of various animal species[60]. These enzymes are also released with the disruption of the mast cell and are scattered in the connective tissue. Thus, in addition to the release of histamine and heparin, and the release of 5-hydroxytryptamine in rodents, and the formation of a SRS, the release of such enzymes may well contribute to the propagation of the anaphylactic reaction in the connective tissue and indeed to the organism as a whole. It seems conceivable that the differences in the anaphylactic pattern in different species are but reflections of the differences in the make-up of the mast-cells.

To sum up, it appears that mast-cell damage occuring in anaphylaxis is due to an antigen-antibody reaction on the surface of cells. This reaction is dependent on temperature, pH value and ionic strength, requires complement and may be inhibited by a number of substances having enzyme-inhibiting properties. These data, which were obtained originally from a study of isolated mast cells correspond closely to observations on the anaphylactic release of histamine from chopped guinea-pig lung. The differences in the anaphylactic pattern in different species may reflect differences in the chemical and enzymic content of the tissue mast cells.

Zusammenfassung. Der Mechanismus der bei der Antigen-Antikörper-Reaktion zustande kommenden Mastzelldisruption wird anhand der neuesten, vorwiegend an isolierten Zellen durchgeführten Untersuchungen diskutiert; dabei wird insbesondere auf jene Befunde, die für einen enzymatischen Vorgang sprechen, eingegangen[64].

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[58] E. P. BENDITT and M. ARASE, J. exp. Med. *110*, 451 (1959).
[59] M. EDER and A. SCHAUER, Beitr. path. Anat. *124*, 251 (1961).
[60] G. G. GLENNER and L. A. COHEN, Nature (Lond.) *185*, 846 (1960).
[61] R. KELLER and G. C. OPPLIGER, Klin. Wschr. *39*, 1028 (1961).
[62] I. MOTA and I. VUGMAN, Brit. J. Pharmacol. *11*, 304 (1956).
[63] J. H. HUMPHREY, in P. GRABAR and P. MIESCHER, *Immunpathologie* (Benno Schwabe, Basel 1959), p. 218).
[64] I am grateful to Dr. J. F. RILEY, Royal Informary, Dundee, for valuable advice and discussion.