Since the skin of guinea-pig differs from that of man in having mosaic pattern of hair growth, it should be understood that these abnormalities are not interpreted as an indication that the pathological picture would be similar in man also. However, information gathered through these experiments has thrown light on our further understanding of the possible types of tissue damage due to insecticides, and has emphasized due caution in their handling[11].

Zusammenfassung. Nach täglicher Applikation of Parathion werden histologisch Veränderungen der Meerschweinchenhaut festgestellt.

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Complex Formation Between Bee Venom Melittin and Extract of Mouse Skin Detected by Sephadex Gel Filtration

We have been interested in the pharmacological properties of the venom of the honey bee (*Apis mellifera*), and of its isolated constituents. Melittin, the major component of the venom, has been studied by several investigators, since its isolation from whole venom by gel filtration[1,2]. The amino acid sequence of this polypeptide has been determined[3], some of its biochemical reactions have been studied in vitro[4], and its antibacterial properties have been recorded[5]. This unique, surface-active, cationic, histone-like moiety has been found to be slightly radio-protective in mice[6], and may be synergistic with the main radio-protective fraction of bee venom phospholipase A[7,8].

The cationic property of melittin partly accounts for its cytotoxicity with respect to mouse bone marrow stem cells, an effect observed in our earlier study[9]. In that work it was concluded that the stoichiometric relationship between the cell surface area and the weight of added melittin, vis-a-vis cytotoxicity of melittin, could be explained by the known charge on the polypeptide. This interaction between melittin and the cell surface raised questions about the ability of melittin to directly enter the body via the subcutaneous injection route. It was also found during this same study[9] that the addition of purified human serum albumin (fraction V) protected bone marrow cells from melittin cytotoxicity. The lack of such protection by other serum fractions suggested the possibility that a complex formed between melittin and fraction V.

The present work was undertaken to answer the question raised by these two observations, namely, whether: 1. melittin on s.c. injection enters the body as such or 2.

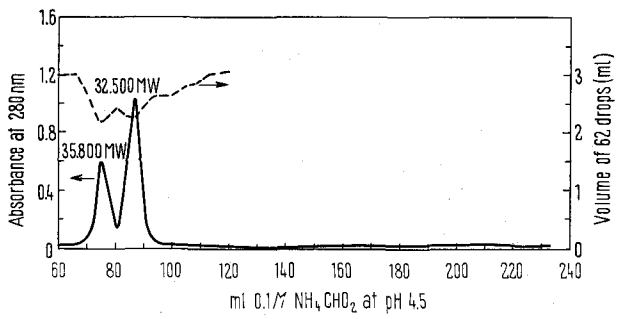

Fig. 1. Separation of the components of human serum albumin (fraction V) on a Sephadex G75-40 column.

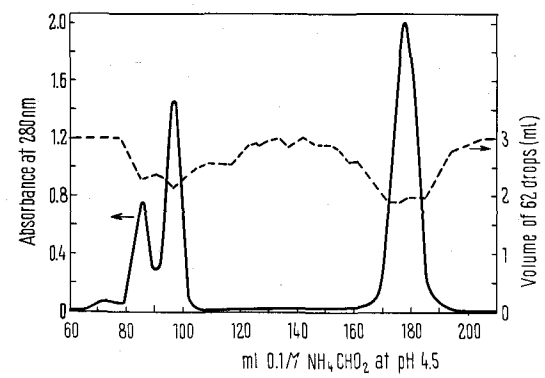

Fig. 2. Separation of the components of a mixture of 27.7 mg human serum albumin (fraction V) and 16.6 mg melittin on a sephadex G75-40 column.

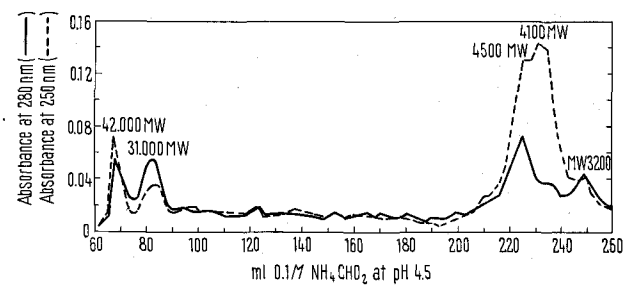

Fig. 3. Separation of the components of mouse skin extract on sephadex G75-40 column.

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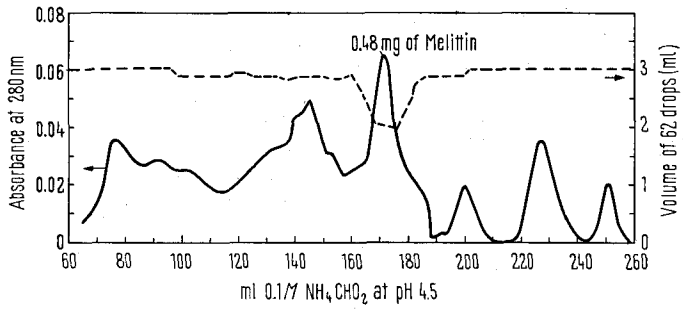

Fig. 4. Separation of the components of a mixture of 2.4 mg melittin and 2 ml of a 10% mouse skin extract on a sephadex G75-40 column.

whether its cationic property first causes it to react with cell surfaces, thereby lysing cells and then entering the body as a complex with the cellular components.

The experimental approach consisted of preparing a mouse skin homogenate, separating the components of the homogenate according to their molecular weights on a Sephadex G75–40 gel-filtration column[2], and then repeating this separation with a mixture of melittin and mouse skin homogenate to detect the presence of a complex. This same methodology was applied to the serum albumin fraction of known concentration and a mixture of melittin and the albumin fraction.

Materials and methods. The melittin used in this study was separated from whole bee venom by gel filtration on a Sephadex G75-40 Column[2]. After isolation, it was lyophilized and stored under refrigeration. The human albumin fraction V was purchased from Sylvana Co., Milburn, New Jersey (Cat. No. 99–213, Lot No. R-25) and stored under refrigeration.

The mouse skin homogenate was prepared by homogenizing 1 g of fresh mouse skin in a 10 Broech glass homogenizer with 10 ml of 0.1*M* NH_4CHO_2 at pH 4.5. The particulate fraction was removed by centrifugation and the supernatant liquid was further clarified by filtration through a 0.45 μm Millipore filter. The resulting clear solution was stored under refrigeration.

To detect the presence of a complex, both the test materials alone and a mixture of the test material and melittin were submitted to gel filtration on a Sephadex G75-40 column. This column was 1×300 cm and was operated at a flow of 0.031 ml, min^{-1}, cm^{-2}, with 0.1*M* NH_4CHO_2 at pH 4.5 as a buffer. The effluent from the column was collected by a fraction collector equipped with a photo-electric drop counter adjusted to collect an aliquot of 62 drops (equivalent to 3 ml buffer) in each tube of the fraction collector. The absorbance, at 280 nm, of the aliquot in each tube was determined after completion of the run.

Results. To characterize the components of human albumin (fraction V), 19.2 mg was dissolved in 1 ml buffer and subjected to gel filtration on the Sephadex G75-40 column. The results can be seen in Figure 1.

The procedure was then repeated with a mixture of 27.7 mg fraction V and 16.6 mg melittin dissolved in 1 ml buffer. The clear solution was placed on the Sephadex G75-40 column and the result can be seen in Figure 2. No evidence for the formation of a complex under these conditions was apparent. The 2 peaks of the albumin are in the same position as in the original compound and the presence of 1 large melittin peak at 168 ml is consistent with the molecular weight of melittin. Therefore, it must be concluded that at pH 4.5 there is no complex formation between melittin and the human serum albumin. The experiment was repeated at pH 7.2 with the same result, as shown in Figure 2.

An hypothesis other than complex formation must therefore be offered to explain the observation that this substance protects bone marrow stem cells from damage by melittin. We suggest that albumin may compete with melittin for the same cell surface receptor sites.

Figure 3 shows the fractions separated from 2 ml of a 10% mouse skin extract. In this sample the absorbance of the aliquot in each tube from the fraction collector was measured at both 280 and 250 nm. This dual measurement facilitates the recognition of proteinaceous material. Only the substance with a molecular weight of 31,000 suggests a protein structure.

When 2.4 mg of melittin was added to 2 ml of the 10% mouse skin extract and the resultant clear solution passed through the Sephadex column, clear evidence for complex formation was found. Figure 4 shows the complexity of the reaction. In this experiment the surfactancy[2] was monitored as well as the absorbance at 280 nm. In order to determine the amount of unreacted melittin present, the total absorbance of the melittin was found by integrating its peak and comparing this number with standard calibration curves. From this data it was found that 0.48 mg was present. It is clear that only the unreacted melittin shows any surface activity. This experiment was repeated using 3.9 mg of melittin and 2 ml mouse skin extract. The results were the same, with 2.2 mg of melittin not reacting. From these 2 experiments it is possible to calculate that 1.8 $\pm$ 0.1 mg of melittin reacted with the 2 ml of the 10% mouse skin extract. This stoichiometry is further evidence of complex formation.

It is apparent from these data, that the probability of melittin entering the body by the subcutaneous route without prior modification of the molecule – such as complex formation – is very low. What changes, if any, occur in the biochemical and pharmacological properties of melittin as a result of the formation of these complexes is still not known. Further studies will be necessary to determine the in vivo pharmacological effects of the melittin complexes.

Zusammenfassung. Das aus Bienengift von *Apis mellifera* gewonnene, oberflächenaktive, kationische Polypeptid Melittin bildet mit Hautzellenextrakt, nicht aber mit Serumalbumin, einen stöchiometrischen Komplex, der vermutlich die Transportform des Giftes im Körper darstellt.

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