sich bei den von uns angewandten IAK-Nachweismethoden nicht von dem Serum der Kontrolltiere, welche niemals Insulin erhielten. Eine Beteiligung der IAK an den Adaptationsvorgängen ist deshalb nicht anzunehmen. Als Ursache für das Fehlen von IAK könnte an die Induktion einer Immunotoleranz[12] durch die einschleichende Insulinapplikation gedacht werden, zumal über die gute immunologische Reaktivität dieser Spezies gegen Insulin[4], insbesondere gegen das therapeutisch beim diabetischen Hund verabreichte identische Insulinpräparat[13], erst kürzlich berichtet wurde. Für das Entstehen einer IAK-bedingten Insulinresistenz ist deshalb ausser den bekannten Faktoren (unter anderem Reaktivität des Individuums, Reinheit des Insulins) auch der Modus der initialen Insulinierung entscheidend. Dies sollte bei der Diskussion über die Ursachen von IAK bei Patienten, wie auch bei der Behandlung der Diabetiker, berücksichtigt werden.

Summary. Subcutaneous injection of exogenous insulin always results in a production of antibodies against insulin. But after a slowly increased dose of insulin (a daily end dose of 3.5–4.2 U/kg) insulin antibodies could not be found in the serum of 2 dogs. This failure of immunogenic action of insulin is discussed as an induction of immunotolerance by the modus of initial antigen application.

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Reduced Lymphatic Drainage from Hamster Cheek Pouch: A Proposed Explanation of its Immunologically Privileged Character

The question of whether or not the cheek pouch of the hamster contains lymph vessels for the egression of foreign antigen to the regional seats of host sensitization in the evocation of an immunological response has come to be a central issue in the elucidation of its immunologically privileged character. While the classical methods of injecting carbon and vital dyes have failed to demonstrate lymphatic channels in the cheek pouch connective tissue or leading therefrom to its regional cervical lymph nodes[1,2], the recent histological verification of lymph vessels in the pouch[3] and of a retarded blackening of the ipsilateral submental nodes after India ink injection to the pouch[4] support the existence of a lymphatic drainage from this organ. By depositing an iron compound into the cheek pouch wall and later histochemically staining the regional lymph nodes for iron at regular intervals, additional evidence is here presented for a delayed and reduced lymphatic drainage from this site.

Eight golden hamsters (*Mesocricetus auratus*), obtained commercially (Hilltop Lab Animals, Scottdale, Pa.), of both sexes and weighing 70–80 g, were anesthetized with sodium amobarbital (100 mg/kg i.p.), their left cheek pouches everted and pinned to a corkboard, and injected with 0.1 ml of a 1:1 water dilution of cadmium-free ferritin (Nutritional Biochemicals Corp., Cleveland, Ohio) into the cheek pouch wall. The pouches were then wiped clean of any excess ferritin and replaced in the hamsters' mouths. 2 hamsters were sacrificed by overdosing with anesthesia at intervals of 2, 4, 6, and 24 h post injection and their left cheek pouches, left and right submental lymph nodes, and livers removed, fixed in 10% neutral formalin, and sectioned in paraplast; hematoxylin-eosin and Gomori's iron stains were performed on all specimens. Another group of 8 hamsters, serving as controls, received 0.1 ml of the ferritin solution intracutaneously to the left part of their upper lips. These animals were sacrificed and their tissues processed as in the cheek pouch group.

The presence of stainable iron in the ipsilateral submental lymph node prior to any systemic dissemination of this compound was considered as indicative of a lymphatic drainage from the cheek pouch. It was found that histochemically demonstrable iron was present only in a few foci of the ipsilateral nodes in the group sacrificed at 24 h post implantation of ferritin in the cheek pouch (Figure A). In comparison, the same nodes already contained a greater amount and distribution of stainable iron at 2 h after injection to the lip (Figure B), but did not appear to increase measurably in quantity during the first 24 h. In both cases, stainable iron was essentially limited to the lymph nodes' sinusoidal system. No iron deposition was found in the livers of the animals of either group.

This reduced and delayed egression of ferritin from the hamster cheek pouch agrees well with our previous results using India ink injected into an isolated cheek pouch chamber assembly[4]. In fact, the reduced drainage by a factor of about 10 is identical with that obtained with carbon[4]. It would seem, then, that molecules ranging from 9 to 13 nm, or their conglomerates, drain from the cheek pouch to its regional ipsilateral submental lymph node at 1/10 the rate as from adjacent areas of the head. This finding thus militates against the view[1] that no direct route exists between the cheek pouch and its regional lymph nodes. Since lymph vessels have been demonstrated in histological sections of the cheek pouch[3], our results might reflect 1. an impeded spread of such particulate matter within the cheek pouch connective tissue thus not bringing a sufficient number of molecules to the lymph channels, in accordance with BILLINGHAM and SILVERS'[5] connective tissue 'barrier' hypothesis, 2. a paucity of lymph vessels in the cheek pouch, and/or 3. lymph vessels in the pouch which are of a different functional character than those elsewhere in the hamster.

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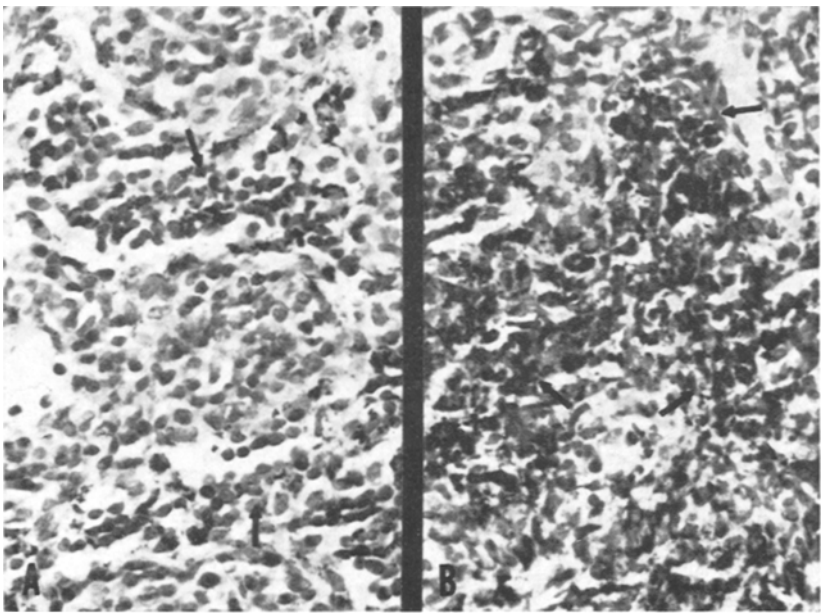

Histological appearance of ipsilateral submental lymph nodes at 24 h after injection of ferritin to hamster cheek pouch (A) and at 2 h after inoculation of hamster lip (B) (Gomori's iron stain, ×450). Black granules represent iron deposits (arrows).

In any case, this situation of a reduced and delayed lymphatic drainage from the hamster cheek pouch affords foreign grafts in this site a decisive advantage for prolonged survival, particularly also because of the availability of a rich and plastic vascular supply[6].

Zusammenfassung. Zur Untersuchung des Lymphabflusses beim Hamster wurde Ferritin in die Backentaschen und Lippen der Tiere injiziert. 2 h nach der Injektion in die Lippe fanden sich grosse Mengen des Ferritins im ipsilateralen submentalen Lymphknoten, während sich nach 24 h und nach Injektion in die Backentasche nur wenig Ferritin fand, was als Zeichen einer immunologischen Bevorzugung der Hamster-Backentasche angesehen wird.

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9 February 1970.

[6] Acknowledgments. I am indebted to Dr. EDWIN R. FISHER for helpful discussions and advice. This work was supported in part by a grant from The Council for Tobacco Research – USA.

Coagulation of Hemolymph of the Larval Honey Bee (*Apis mellifera* L.)

Previous investigations in the field of invertebrate blood, or hemolymph, coagulation have consisted primarily of morphological description rather than identification of the chemical substances involved in the clotting process. For example, GREGOIRE[1-3] studied patterns of blood coagulation in various insects by using phase-contrast microscopy. The purpose of this investigation was to determine whether the hemolymph of the honey bee, *Apis mellifera* L., contains clotting factors similar to those found in human blood and why the hemolymph does not coagulate.

Hemolymph was obtained by gently puncturing larvae of various ages with a sterile hypodermic needle and drawing the fluid which exuded from the wound into a capillary pipette. The hemolymph was pooled in shell vials and stored at – 20 °C. (Blood of pupae and adults was not used because of the difficulty of obtaining sufficient quantities.) This pooled larval honey bee hemolymph was then tested for the presence of the blood coagulants that exist in human blood.

Normal plasma and serum obtained from human volunteers were used as standards. Insect hemolymph was treated in exactly the same manner as human blood. The following determinations for coagulants were made in triplicate on each sample using the methods of EICHELBERGER[4]:

1. One-stage prothrombin time.
2. Partial thromboplastin time.
3. Thromboplastin generation test.
4. Thrombin clot time.
5. Antihemophilic globulin (Factor VIII).
6. Plasma thromboplastin antecedent (Factor XI).
7. Recalcification time and nonspecific mixing test for anticoagulants.
8. Proaccelerin (Factor V).
9. Proconvertin (Factor VII).
10. Plasma thromboplastin component (Factor IX).
11. Hageman factor (Factor XII).
12. Stuart factor (Factor X).

The results of the coagulation tests are given in Tables I and II and in the Figure. The clotting times of honey bee hemolymph in each test were at least 2–3 times as long as that of human plasma. The absence of proconvertin from the hemolymph prevented the formation of a clot in the test of one-stage prothrombin time.