borohydride, while both groups are reduced by lithium aluminum becomes explicable, if one assumes that formation of a carbonyl-metal complex is necessary for the reduction of the less polar ester group.

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Zusammenfassung

Die gegenwärtig akzeptierte Theorie des Mechanismus der Reduktion von Ketonen mit Metallhydriden wird diskutiert. Verschiedene Unstimmigkeiten werden aufgezeigt, und ein neuer Mechanismus wird vorgeschlagen, der mit den experimentellen Befunden besser übereinstimmt.

Enhancement of Antibody Formation by Hypersensitivity Reaction

It was investigated in young animals whether the formation of antibodies is accelerated or enhanced by injection of endotoxin shortly after birth[1]. Similar results were obtained as in the experiments in which vitamin B_{12} was administered[2]: such pretreatments of the young animals did not affect antibody production but significantly increased their resistance to infection. Attempts were therefore made to elucidate why endotoxins do not influence antibody formation in young animals; injected simultaneously with protein antigens to adult animals, they cause a marked increase in antibody response[3]. We assume that the stimulating action of endotoxin in the adults may be due to the hypersensitivity reaction[4]. This reaction elicited by endotoxin is, however, hardly to be distinguished from its direct toxic action on tissue metabolism. Pure protein antigens were therefore used in order to demonstrate the significance of the hypersensitivity reaction for enhancement of antibody formation.

Rabbits (weight 2–3 kg) were sensitized by injection of 10 mg of human serum albumin (HSA) in lipoid adjuvant into the foot-pads. After four weeks, when high immune response was developed in the sensitized rabbits and intradermal injection of HSA elicited an intense skin test (immediate and delayed type of hypersensitivity were present), the experimental animals were divided in three groups: (1) HSA sensitized animals were given intravenously a challenge dose of HSA (30 μg) simultaneously with a small first dose (10 μg) of ovalbumin (OA). (2) HSA sensitized animals were injected intravenously with ovalbumin (10 μg) only. (3) Normal non-sensitized rabbits received the same amount of HSA (30 μg) + OA (10 μg) as the first group of sensitized animals. The blood from injected animals was collected at intervals of two days and with the sera hemagglutination was carried out using tannic acid treated blood cells coated with HSA or OA[5]. For hemagglutination the micro-method of TAKÁTSY was used[6].

Formation of antibodies to the second antigen – ovalbumin – was demonstrated in the first experimental group, i. e. in the HSA sensitized rabbits which were injected simultaneously with HSA and OA (Fig. 1). The same amount of HSA and OA injected into the control (non-sensitized) rabbits did not give rise to the formation of antibodies to ovalbumin (Fig. 2). That these different

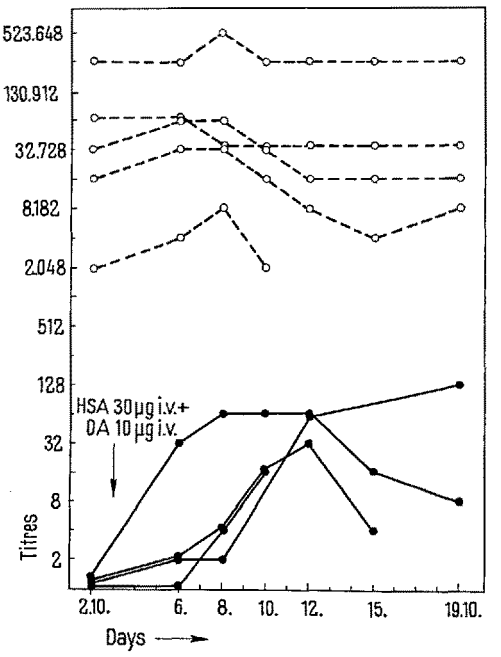

Fig. 1. Antibody titres determined by hemagglutination in the group of HSA sensitized rabbits injected i. v. with 30 μg HSA + 10 μg OA; anti-OA titre – full line, anti-HSA titre – dashed line.

results cannot be explained on the base of secondary response as a cross reaction between HSA and OA is evident from the following findings:

In the rabbits sensitized with HSA and showing anti-HSA titres of thousands, no antibodies to ovalbumin have been demonstrated. When the animal having anti-HSA and anti-OA titre was injected with 30 μg of HSA, only the level of anti-HSA and not anti-OA was affected (Fig. 4). If the HSA sensitized rabbits received injection of OA (10 μg) only, they did not form a demonstrable amount of antibodies to OA (Fig. 3). This also provides evidence that the formation of antibodies to ovalbumin in sensitized animals is not caused by non-specific stimulation of mesenchymal tissue, e. g. by increasing the amount of antibody-forming cells. On the contrary, it may be concluded that stimulation of the antibody response occurred only when the dose of the second antigen (OA) was injected simultaneously with the challenge dose of HSA, i. e. when the hypersensitivity reaction was elicited. Substances released in the course of hypersensitivity reaction might be responsible for the increase in antibody response.

A similar result, i. e. enhancement of antibody formation in sensitized animals, was achieved by GOOD *et al.*[7]. Animals were sensitized with BCG vaccine and hypersensitivity reaction elicited by injection of old tuberculin.

[1] J. ŠTERZL, M. HOLUB, and I. MILER, Folia microbiol., in press (1960).

[2] J. ŠTERZL, Z. TRNKA, and M. HOLUB, Folia microbiol. *4*, 298 (1959).

[3] A. G. JOHNSON, J. GAINES, and M. LANDY, J. exp. Med. *103*, 225 (1956). – R. M. CONDIE, S. J. ZAK, and R. A. GOOD, Proc. Soc. exp. Biol. Med., N. Y. *90*, 355 (1955). – P. KIND and A. G. JOHNSON, J. Immunol. *82*, 415 (1959).

[4] CH. A. STETSON, J. exp. Med. *101*, 421 (1955).

[5] A. B. STAVITSKY, J. Immunol. *72*, 360 (1954).

[6] G. TAKÁTSY, Acta microbiol. Acad. Sci. Hung. *3*, 191 (1955).

[7] R. A. GOOD, R. M. CONDIE, G. THOMPSON, and D. R. JENSEN, Fed. Proc. *16*, 178 (1957).

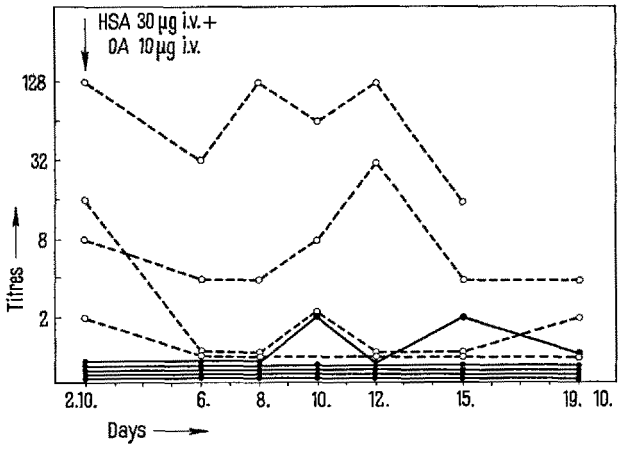

Fig. 2. The group of non-sensitized rabbits injected i. v. with 30 µg HSA + 10 µg OA; anti-OA titre – full line, anti-HSA titre – dashed line.

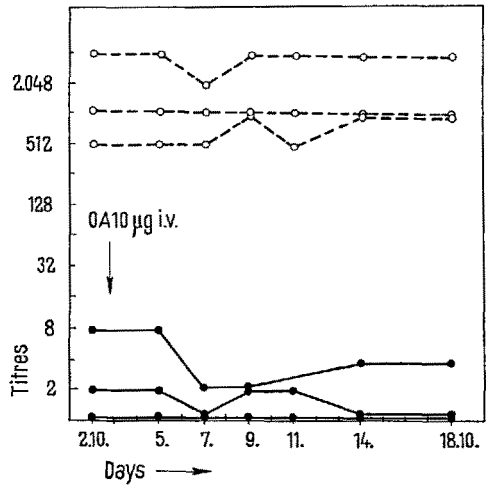

Fig. 3. HSA sensitized rabbits were injected i. v. with 10 µg of OA only; anti-OA titre – full line, anti-HSA titre – dashed line.

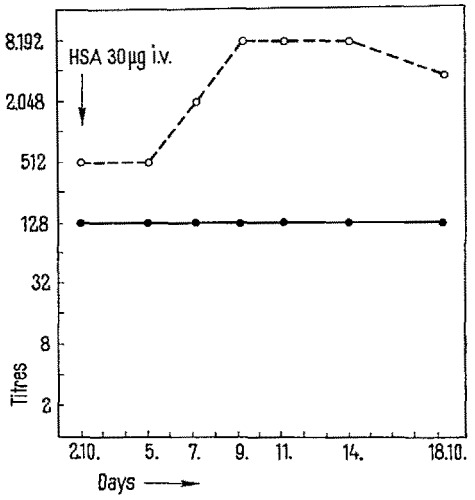

Fig. 4. The rabbit with anti-OA and anti-HSA titre was injected with 30 µg of HSA; anti-OA titre – full line, anti-HSA titre – dashed line.

In that experiment the hypersensitivity might not have been the only cause of enhancement of antibody response since in old tuberculin materials possessing certain of the properties of endotoxin have been demonstrated[8]. Our experiments, in which the hypersensitivity reaction elicited by non-detrimental protein antigens enhanced the antibody response, may support the hypothesis advanced by STETSON[4] that the stimulating action of endotoxin is probably due to the hypersensitivity reaction provoked by this substance. In young animals, therefore, which do not develop hypersensitivity to endotoxin, the antibody response is not enhanced. Another evidence of the absence of hypersensitivity reaction in young animals is the tolerance of significantly higher amount of injected endotoxin than in adults[9].

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Zusammenfassung

Eine Anzahl Kaninchen wurde durch humanes Serumalbumin (HSA) sensibilisiert. Wurde bei ihnen durch eine weitere Dosis desselben Antigens (HSA) eine Hypersensitivitätsreaktion hervorgerufen, so wurde die Antikörperbildung gegen ein anderes gleichzeitig eingeführtes Antigen (Ovalbumin-OA) stimuliert.

[8] CH. A. STETSON, S. SCHLOSSMAN, and B. BENACERRAF, Fed. Proc. *17*, 536 (1958).

[9] J. H. PARISH and C. C. OKELL, J. Path. Bact. *33*, 327 (1930). – P. A. ZAHL, S. H. HUTNER, and F. S. COOPER, Proc. Soc. exp. Biol. Med., N. Y. *54*, 137 (1943). – L. THOMAS, Ann. Rev. Physiol. *16*, 467 (1954).

PRO EXPERIMENTIS

Ein einfaches Verfahren zur Sichtbarmachung von Glasmikroelektroden mit Hilfe von Fluorescein

Bei Verwendung von Glasmikroelektroden nach dem bekannten Verfahren von LING und GERARD[1] zur intrazellulären Ableitung von Ruhe- und Aktionspotentialen ist es meist relativ schwierig, den Einstichort genau zu lokalisieren. Dies ist grösstenteils dadurch bedingt, dass die dünnen, mit 3 *M* KCl-Lösung gefüllten Glaskapillaren nahezu parallel zum Strahlenverlauf des Beobachtungsmikroskopes angeordnet sind; aus diesem Grunde und zum Teil auch wegen Auftretens von störenden Reflexen ist es nicht immer möglich, die Mikroelektroden bis an ihre Spitzen zu verfolgen. Eine Lokalisation des Einstiches ist aber namentlich dann erforderlich, wenn der genaue Abstand zwischen Reiz- und Mikroelektroden bzw. zwischen zwei Mikroelektroden, beispielsweise zur Bestimmung der Leitungsgeschwindigkeit, mit Hilfe eines Okular-Netzmikrometers festgelegt werden soll.

Eine verhältnismässig einfache Methode zur besseren Sichtbarmachung von Glasmikroelektroden sei im folgenden kurz beschrieben: Die mit der Hand oder durch eine Elektroden-Ziehmaschine hergestellten Mikrokapil-

[1] G. LING and R. W. GÉRARD, J. cell. comp. Physiol. *34*, 383 (1949).