Tab. I. The effect of litter rank on lymph node development in C57BL/Sp mice[a]

Age weeks	No. of mice	Body weight g	Lymph node weight mg
		First litter	
3	14	6·2 (0·4)	6·0 (0·5)
6	12	15·1 (0·3)	13·9 (1·0)
Increase – %		144	132
		Second litter	
3	26	6·8 (0·3)	6·2 (0·5)
6	27	14·5 (0·3)	13·1 (0·7)
Increase – %		113	111
		Third litter	
3	12	5·8 (0·4)	5·3 (0·7)
6	29	13·1 (0·4)	9·3 (0·7)
Increase – %		126	76
		Fourth litter	
6	9	12·2 (1·2)	9·9 (1·4)

[a] Values are means and their standard errors.

Tab. II. The effect of litter rank on lymph nodes of C3H/AnSp and AKR/Jax mice[a]

Strain	No. of mice	Body weight g	Lymph node weight mg
		First litter	
C3H	12	15·3 (0·5)	21·1 (1·3)
AKR	10	14·1 (0·7)	10·7 (0·6)
		Second litter	
C3H	7	13·2 (0·6)	17·2 (1·1)
AKR	9	14·9 (0·6)	14·6 (1·7)

[a] Values are means and their standard errors.

Work with germ-free animals[7] has indicated that in the absence of an outside stimulus lymphatic tissue fails to develop to an appreciable extent. The present experiments suggest that some maternal stimulus in C57BL/Sp mice apparently diminishes with the age of the mother and/or the number of litters raised.

The effect of litter rank on spontaneous tumor incidence has not been found to be uniform. Bittner[2] observed that mammary tumor incidence was increased in advanced litters. On the other hand, MacDowell *et al.*[4] found a decrease in leukemia incidence in later litters. Preliminary experiments (Table II) indicated that the effect of litter rank on nodes from mice with a high incidence of either spontaneous mammary cancer (C3H/AnSp) or leukemia (AKR/JaxSp) was likewise not uniform. Lymph nodes from 6–$7^1/_2$-week old second litter C3H/AnSp mice were smaller than those of age matched first litter mice; while nodes from 6–$7^1/_2$-week old second litter AKR/JaxSp mice were larger than those of age matched first litter mice. These differences were significant at the 5 percent level. These preliminary findings suggest the possibility that alteration in the quantity of the lymphatic component of host defense may be involved in the alteration of spontaneous tumor incidence by litter seriation.

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Zusammenfassung

Das Gewicht der Lymphknoten von 6–$7^1/_2$ Wochen alten C57BL- und C3H-Mäusen sank in aufeinanderfolgenden Würfen ab. Umgekehrte Verhältnisse fanden sich bei AKR-Mäusen. Verzögertes Wachstum war für die kleinen Lymphknoten der späteren Würfe bei drei und sechs Wochen alten C57BL-Tieren verantwortlich.

DISPUTANDUM

The Stereochemistry of Reduction by Complex Metal Hydrides

It has been concluded that two effects operate to determine the stereochemistry of an alcohol obtained by hydride reduction of an asymmetric cyclohexanone: the ease of formation of the complex between the carbonyl group and the complex hydride (steric approach control); and the relative energetics of the formation of the products once the initial complex is produced (product development control)[1]. While there is some disagreement over the exact proportions of epimeric alcohols obtained in the reductions[2,3], it seems clear that as Dauben *et al.* originally reported, use of sodium borohydride as reducing agent tends to lead to a greater proportion of the unstable epimeric alcohol than does the use of lithium aluminum hydride. Dauben suggests that this difference in the composition of products is due to the greater effective size of the borohydride species as compared with lithium aluminum hydride.

Although this hypothesis appears to have been generally accepted[4], there are difficulties associated with it. The B-H bond length in borohydride is 1.25 A[5] and it has also been found from measurements of force constants that the Al-H bond length in the aluminum hydride ion is 1.66 A[6]. These bond-length values show that the correctness of Dauben's suggestion depends on the borohydride ion in methanol or pyridine being solvated to a greater extent than the aluminum hydride ion in ether or tetrahydrofuran.

While the theory of ion-solvent interactions is not yet in an entirely satisfactory state, it is thought that monovalent anions which have a crystal radius of 1.80 A or greater are not solvated in water or methanol[7]. The crystal radius of the borohydride ion is 2.03 A[8] and the

We thank Dr. D. J. Royer of the Georgia Institute of Technology, Dr. W. R. Gilkerson of the University of South Carolina, and Dr. R. H. Harris of the University of Nebraska for many helpful discussions concerning the physical and inorganic chemistry of the complex metal hydrides.

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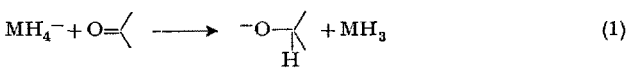
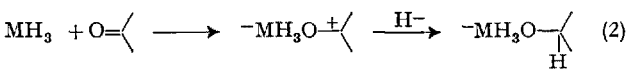
radius of the aluminum hydride ion should be about 0.4 A larger, which suggests that neither the borohydride ion nor the aluminum hydride ion should be solvated. Such experimental evidence as is available tends to support this view, e. g., D'OR and FUGER[9] concluded from studies of infrared spectra that aluminum hydride ions do not complex with ether.

As DAUBEN's explanation of the difference in amounts of epimeric alcohols obtained by borohydride and aluminum hydride ion reduction is not supported by present solvation theory this alternative is proposed: We suggest that in the reductions of carbonyl groups by complex metal hydrides, two reactions are possible; either a direct attack on the carbonyl by a metal hydride anion (1), giving a relatively large proportion of unstable alcohol, owing to the preferred approach of the hydride ion from the less



(1)



(2)

hindered (equatorial) side of the molecule, or the formation of a complex between the metal hydride and carbonyl (2) which adopts the more stable conformation, and is then reduced. We also suggest that this difference in the course of the reductions is primarily due to the chemical nature of the reducing agent rather than any solvent effects; this is borne out by the results obtained by the reduction of Δ^8-lanosten-3-one (Tab.).

From a consideration of DAUBEN's hypothesis, one would expect that lithium borohydride in nonpolar solvents would afford about the same or perhaps more equatorial alcohol than does lithium aluminum hydride. However, this does not appear to be the case, for lithium borohydride reduction of lanostenone gives about the same proportion of equatorial isomer as is obtained with the other borohydrides. This similarity of lithium borohydride and the other borohydrides has also been noted in the reduction of tropinone[12]. In addition, lithium tritertiarybutoxyaluminum hydride which is larger than lithium aluminum hydride gives more of the equatorial isomer than does lithium aluminum hydride[10].

It seems likely that lithium aluminum hydride, and the substituted lithium aluminum hydrides, have open to them two modes of dissociation, either a direct ionization[9,13] to lithium ions, and aluminum hydride ions, or a homolytic cleavage to lithium hydride and aluminum hydride (3)[14].

$$LiH + AlH_3 \longleftarrow LiAlH_4 \longrightarrow Li^+ + AlH_4^- \qquad (3)$$

$$MBH_4 \longrightarrow M^+ + BH_4^- \quad M = Li, Na, K \qquad (4)$$

The AlH_3 is capable of behaving as a Lewis acid and may complex with the carbonyl group; the hydride ion is then delivered by any of the appropriate species in solution. The composition of alcohol mixtures obtained by reductions with lithium aluminum hydride varies from the equilibrium mixture obtained using lithium aluminum hydride/aluminum chloride with acetone present[15,16]. This shows that there must be a certain amount of direct attack of AlH_4^- without the preliminary complexing. The greater proportion of stable isomers formed by $LiAl(O\text{-}t\text{-}Bu)_3H$[10] is explained by the tendency of the bulky tritertiarybutoxy aluminum group to take up an equatorial position.

Reducing Agent	%Δ^8-lanosten-3β-ol
$LiAl(O\text{-}t\text{-}Bu)_3H$/tetrahydrofuran[10]	100
$LiAlH_4$/ether[11]	88
$LiAlH_4$/tetrahydrofuran[11]	90
$LiBH_4$/tetrahydrofuran[11]	70
$NaBH_4$/isopropyl alcohol[11]	77
KBH_4/isopropyl alcohol[11]	80

In the case of the considerably more ionic borohydrides[8,17,18] it may be assumed that dissociation occurs to give predominantly metal ion and borohydride ion (4), the direct attack of which will afford more of the unstable epimer than is obtained using lithium aluminum hydride.

In the reduction of a hindered ketone the delivery of hydride from the axial direction is retarded and so a predominance of axial alcohol is obtained when either borohydride or aluminum hydride is used. However, here again the considerations presented above suggest that the percentage of axial alcohol obtained will be greater when borohydride is the reagent.

One special class of hindered ketones involves cases (quoted by DAUBEN[1]) in which a ketone is reduced by lithium aluminum hydride but not by sodium borohydride. DAUBEN uses two such examples, methyl b-oxopolyporenate A a-acetate and a 9-bromo-11-oxosteroid, as evidence for his suggestion that the borohydride ion is larger than the aluminum hydride ion. These results can be rationalised in terms of our proposals. Both of the compounds mentioned by DAUBEN contain not only β-axial methyl groups (which hinder the approach of reagents from the β side) but in addition have large substituents (bromine or methyl) in α-axial orientations. This means that attack on the molecule from the α side is also hindered, and is probably the reason sodium borohydride does not reduce these compounds. In contrast, lithium aluminum hydride can complex with the oxygen atom of the carbonyl group, and thus activates the group so that it attracts in a hydride ion despite the steric hindrance. The hydride ion enters along the less hindered path available; hence, an axial alcohol is obtained.

In addition to explaining the stereochemistry of the products obtained by the reduction of various ketones by metal hydrides, our hypothesis rationalises the differences in reactivity of the various metal hydrides towards certain classes of organic compounds. For example, the well-known selective reduction of ketones in the presence of ester groups by sodium and potassium

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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

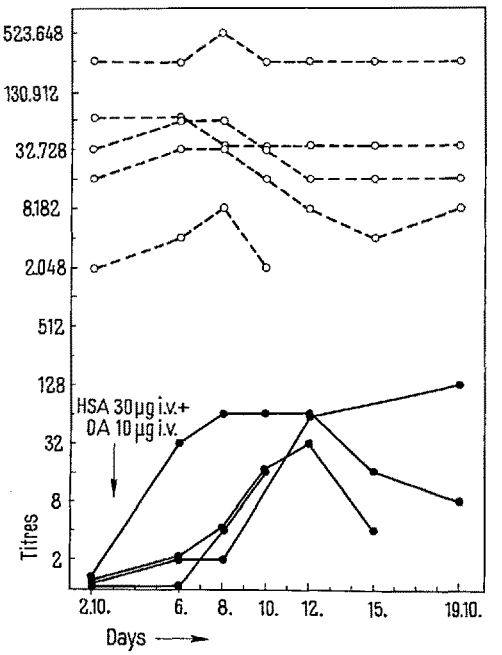

Fig. 1. Antibody titres determined by hemagglutination in the group of HSA sensitized rabbits injected i. v. with 30 μg HSA + 10 μg OA; anti-OA titre – full line, anti-HSA titre – dashed line.

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.

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