ratio of indigestible wooden blocks to pellets retrieved during stimulation was only 0.83 percent, a figure not significantly different from the proportion retrieved during deprivation (0.54 percent).

This experiment indicates that hoarding of food can be produced by electrical stimulation of those drive mechanisms in the hypothalamus which normally give rise to eating. Three theories of hoarding have been briefly referred to. The present result is evidence against a mechanism unrelated to physiological drive; it is also evidence against a nonspecific arousal effect manifesting as increased hoarding, since the control group showed exploratory behavior and other signs of response to stimulation, but no increase in hoarding. The result supports the supposition, implicit in the depletion hypothesis, that hoarding is brought about by a mechanism concerned in the regulation of body weight (*1, 2*). The experiment also throws light on how this mechanism, the lateral hypothalamic feeding area, monitors the nutritional requirements of the body. It has been suggested that the normal activity of the feeding area is spontaneous in origin and subject only to a braking action exerted by the hypothalamic ventromedial nucleus, the latter, in turn, being activated by the ingestion of food (*7*). However, since food ingestion inhibits further feeding but not hoarding (*1, 2*), it appears that the ventromedial nucleus acts only on certain efferent pathways from the lateral hypothalamus that subserve feeding, not on the lateral hypothalamus as a whole. This conclusion is consistent with duplex theories of hunger regulation which distinguish two separate regulatory mechanisms: a short-term control by the ventromedial nucleus, and an independently varying long-term regulation mediated by a proposed chemoreceptive mechanism in the lateral hypothalamus (*8*). The lateral hypothalamic mechanism, in responding to slow changes in metabolic demand, appears to be responsible both for the occurrence of eating in the nonsatiated animal, and regardless of activity in the ventromedial nucleus, for the motivation of hoarding.

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References and Notes

1. C. T. Morgan, E. Stellar, O. Johnson, *J. Comp. Psychol.* **35**, 275 (1943).
2. J. B. Wolfe, *ibid.* **28**, 97 (1939).
3. J. Mayer, *Ann. N.Y. Acad. Sci.* **63**, 15 (1955).
4. C. T. Morgan, *Psychol. Rev.* **54**, 335 (1947).
5. D. O. Hebb, *ibid.* **62**, 243 (1955); F. Glover, thesis, London University, 1961.
6. N. E. Miller, *Science* **126**, 1271 (1957); E. E. Coons, M. Levak, N. E. Miller, *ibid.* **150**, 1320 (1965).
7. J. Mayer and S. Sudsaneh, *Amer. J. Physiol.* **197**, 274 (1959).
8. J. A. Deutsch, *The Structural Basis of Behaviour* (Cambridge Univ. Press, New York, 1960), pp. 23–34; L. J. Herberg, *Quart. J. Exp. Psychol.* **14**, 8 (1962).
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Mechanism of Delayed Reactions

Abstract. *Arsanilic acid conjugates of polymers of* L-*typrosine, glutamic acid, and alanine are immunogenic and can elicit hapten-specific, delayed-hypersensitivity reactions in sensitized guinea pigs. Conjugates of the* D-*amino acid polymers are neither immunogenic nor capable of eliciting delayed reactions. Mixtures of small amounts of conjugates capable of eliciting a delayed reaction with larger amounts of* D-*amino acid polymer conjugates produce only small delayed reactions. I suggest that the delayed reaction is an active response requiring the continued participation of immunogenic material in sensitized animals; it is not the reaction of preformed antibody-like material with the antigenic determinant.*

The discovery of hapten-specific delayed hypersensitivity produced by conjugates of arsanilic acid (*1*) has provided a useful model system for comparing various aspects of delayed sensitivity and antibody synthesis and reaction. In studies concerned with specificity it was found that, while guinea pigs could be sensitized with certain conjugates of arsanilic acid and poorly antigenic carriers, the delayed reactions could be elicited with virtually any conjugate (*2*).

These findings suggested that, as with antibody, the specificity of these reactions was directed toward the azobenzenearsonate group, which was effective on almost any unrelated carrier. In the course of these studies, several polymers of D-amino acids became available; their conjugates were therefore compared with those made from the corresponding L-amino acids regarding their ability to sensitize and elicit delayed reactions. The work I now report confirms other results that indicate that the conjugates of D-amino acid polymers are not antigenic (*3, 4*) when administered alone in Freund adjuvant.

Since such conjugates of D-amino acid polymers can elicit anaphylactic reactions with antibody (*3*), their failure to elicit delayed reactions in sensitized animals suggests that delayed hypersensitivity is not a passive reaction of the appropriate antigenic determinant with a preformed sensitizing moiety, akin to antibody reactions, but that it entails the active participation of a conjugate that is per se antigenic, much as in a secondary antibody response.

The conjugates used by me were prepared and purified, in a manner described (*1*), by coupling, at *p*H 8 to 9, overnight with diazotized arsanilic acid and by precipitation in acid. Conjugation was in the proportion of 10^{-5} mole of arsanilic acid per 10 mg of carrier. Samples of poly-L- and poly-D-glutamic-alanine-tyrosine (poly-L-GAT and poly-D-GAT) were donated by Paul Maurer, poly-D-tyrosine (poly-D-T) and poly-L- and poly-D-glutamic tyrosine (poly-L-GT and poly-D-GT) were donated by Michael Sela, and poly-L-tyrosine (poly-L-T) was purchased from New England Nuclear Corporation, Bedford, Massachusetts.

White, male, 400-g guinea pigs were injected in the four foot pads, each with a total of 0.1 ml of complete adjuvant containing 10^{-6} mole of the azobenzenearsonate conjugate of either D- or L-*N*-acetyltyrosine (ABA-tyr). Two weeks later they were shaved, depilated, and tested with the appropriate antigens injected intradermally in 0.1 ml of saline. Skin sites were examined after 3 hours for evidence of Arthus reaction and again after 24 hours for delayed reactions.

Previous study (*5*) had shown that guinea pigs immunized with either D- or L-ABA-tyr monomer uniformly developed delayed sensitivity to conjugates of guinea pig–serum albumin or poly-L-GAT, but not to poly-D-GAT. In order to ensure that the failure to produce skin reactivity with the D-polymer conjugate was not an artifact due to insufficient coupling or poor solubility, the experiment was repeated and enlarged to study the development of hapten-specific delayed sensitivity with several sets of D- and

L-polymers; the results (Table 1) confirm and enlarge these observations.

All guinea pigs, whether immunized with D- or L-ABA-tyr, developed similar delayed reactions to the ABA conjugates of poly-L-T, poly-L-GAT, and poly-L-GT. None of the animals showed delayed reactions to any of the D-polymer conjugates. The reactivity of conjugates of the L-polymers in animals immunized with ABA-D-tyr indicated that the optical activity of the tyrosine residue was not implicated in the specificity of the reaction, and that the reactive entity appeared to be the ABA group and perhaps the aromatic ring to which it was attached. However, since this same grouping was present on the conjugates of the D-polymers, its failure to react was intriguing.

One possibility considered was that immunogenically active material was needed to initiate the delayed reaction, but that, once started, its progression could be ensured by presence of the ABA group on an inert carrier. To test this conception, groups of animals immunized with ABA conjugates of D- or L-tyrosine were tested with similar doses of the D-conjugates to which had been added small priming doses of ABA–bovine serum albumin (BSA).

The results (Table 2) show that such mixtures resulted in delayed reactions little different from that produced by the small dose of ABA-BSA alone. Since it was possible that ABA-BSA was not a suitable initiator for reactions to the amino acid polymers, a similar experiment was performed in which small admixtures of the homologous ABA-L-polymer were made to the test dose of ABA-D-polymer. The results (Table 3) show that the delayed reactions produced by the mixtures were indistinguishable from those produced by the small amount of L-conjugate alone.

Delayed reactions have been shown to originate with the infiltration of a few specifically sensitized cells (*6*), which is followed by a larger outpouring of normal mononuclear cells (*7*) that leads to the characteristic picture of induration and erythema. The mechanism of the interaction between the sensitized lymphoid cells and the antigen remains unknown, although several suggestions have been advanced.

One general category of reaction mechanisms involves antibody functioning at a distance from the cell where it is synthesized. This antibody could be of a special type in terms of affinity (*8*), synthesized by the specifically sensitized cells at the site of the reaction and operating only in the limited area of the reaction; or antibody synthesized more distantly and coming to the site of reaction by way of the circulation. In either event, one would expect such antibody to have the same general characteristics as classic circulating antibody, in possessing the ability to react with the specific antigenic determinant upon contact. In at least one instance (*3*), antibody to the azobenzenearsonate group, produced by immunization with ABA-poly-L-GAT, has been shown to react with the ABA group on poly-D-GAT to give a passive cutaneous anaphylactic reaction. Similarly, antibody to the ABA group can react in vitro or in vivo with the R'3 dye (*9*), which is a trivalent azobenzenearsonate conjugate of resorcinol.

In both these examples the materials that can react with preformed antibody are themselves nonimmunogenic. It is the difference in behavior of these materials in respect to delayed sensitivity and to reaction with antibody that leads us to discard the concept that delayed sensitivity is due to a reaction with formed antibody. Presumably, these considerations should also be valid for antibody bound to a lymphoid cell. Since all the D-polymer carriers used by me have been shown to be nonimmunogenic under conditions such that the L-polymer can sensitize to the attached ABA group, one is led to the conclusion that immunogenicity is somehow implicated in the genesis and development of the delayed reaction.

The observation that small amounts of antigenically active material added to the inactive skin-test material do not lead to significant delayed reactions suggests further that the role played by antigenically active material in a delayed reaction is a continuous one. The degree of the delayed reaction is directly proportional to the amount of ABA-L-polymer added, and the larger amount of ABA-D-polymer present is unable to augment or continue the small reaction initiated.

These results are most consistent with the interpretation that a delayed reaction is an active continuing process between lymphoid cells and material that is inherently immunogenic in that it, itself, can sensitize an animal under appropriate immunizing conditions. The situation most closely resembles an anamnestic or "booster" response in which cells once exposed to antigen

Table 1. Delayed reactions to ABA conjugates of D- and L-amino acid polymers in guinea pigs immunized with ABA-D- or ABA-L-*N*-acetyltyrosine. Six animals were immunized in each way.

Immunization (10^{-6} mole)	Delayed reaction (mm diam.) to 20 μg of ABA-poly-					
	L-tyr	D-tyr	L-GAT	D-GAT	L-GT	D-GT
ABA-L-tyr	7	0	8	0	11	0
ABA-D-tyr	9	0	14	0	15	0

Table 2. Delayed reactions to mixtures of ABA-BSA and ABA-D-amino acid polymers in guinea pigs immunized with ABA-D- or ABA-L-*N*-acetyltyrosine. Six animals were immunized in each way.

Immunization (10^{-6} mole)	Delayed reaction (mm diam.) to ABA-BSA (2 μg) plus 20 μg of			
	Nil	ABA-poly-D-tyr	ABA-poly-D-GAT	ABA-poly-D-GT
ABA-L-tyr	5*	†	†	4
ABA-D-tyr	8*	5	9*	**7**

* Erythema only; no induration. † Slight erythema.

Table 3. Delayed reactions to mixtures of ABA conjugates of D- and L-amino acid polymers. Six animals were immunized in each way.

Immunization (10^{-6} mole)	Delayed reaction (mm diam.) to ABA-L-			
	GT (2 μg)	GT (2 μg) + ABA-D-GT (20 μg)	GAT (2 μg)	GAT (2 μg) + ABA-D-GAT (20 μg)
ABA-L-tyr	4	5	4	4
ABA-D-tyr	**7**	**7**	5	**6**

undergo a rapid proliferative and productive process after reexposure to the antigen. The delayed reaction may, therefore, be the analogue in the skin of the proliferative burst of cellular activity in the lymph node after reinjection of antigen.

This interpretation was suggested by the work of Schlossman *et al.* (*11*); it supports their findings made with an entirely different system. With α-dinitrophenol conjugates of lysine peptides, they found that only conjugates capable of acting as immunogens in delayed sensitivity could elicit delayed reaction. In their case, this property was a function of size, conjugates of the octamer and nonamer being capable of eliciting a delayed reaction while smaller ones could elicit only Arthus reactions. Correspondingly, only the larger conjugates could sensitize a guinea pig to delayed sensitivity. The authors interpreted this delayed reaction as being "analogous to a local secondary response wherein immunogenic peptides can stimulate sensitized lymphocytes to produce cell-bound or free antibody."

The requirement of an immunogenic material to provide a delayed reaction in both sets of experiments makes it seem unlikely that delayed reactions can be attributed to preformed antibody of any sort. Rather the results make it appear likely that the delayed reaction is an active stimulatory process in a sensitized animal reexposed to antigenic material; this second exposure may or may not lead to conventional antibody as a secondary event. Current work on in vitro blast-cell transformations (*12*), and on inhibition of macrophage migration (*13*) in the presence of antigen, suggests that these phenomena are related to delayed sensitivity and are also proliferative or stimulatory reactions. It will be interesting to see whether immunogenic materials are necessary for these reactions also.

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References and Notes

1. S. Leskowitz, *J. Exp. Med.* **119**, 291 (1963).
2. V. E. Jones and S. Leskowitz, *Nature* **207**, 596 (1965).
3. B. Benacerraf, A. Ojeda, P. H. Maurer, *J. Exp. Med.* **118**, 945 (1963).
4. F. Borek, Y. Stupp, S. Fuchs, M. Sela, *Biochem. J.* **96**, 577 (1965).
5. S. Leskowitz, V. E. Jones, S. J. Zak, *J. Exp. Med.* **123**, 229 (1966).
6. J. S. Najarian and J. D. Feldman, *ibid.* **118**, 341 (1963).
7. R. T. McCluskey, B. Benacerraf, J. W. McCluskey, *J. Immunol.* **90**, 466 (1963).
8. F. Karush and H. N. Eisen, *Science* **136**, 428 (1962).
9. L. Pauling, D. Pressman, D. H. Campbell, C. Ikeda, M. Ikawa, *J. Amer. Chem. Soc.* **64**, 2994 (1942); D. H. Campbell and G. E. McCasland, *J. Immunol.* **49**, 315 (1944).
11. S. Schlossman, S. Ben-Efraim, A. Yaron, H. A. Sober, *J. Exp. Med.* **123**, 1082 (1966).
12. J. Mills, *J. Immunol.* **97**, 239 (1966).
13. J. R. David, *J. Exp. Med.* **122**, 1125 (1965).
14. Supported by PHS grant AI-01289.

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Amphibian Orientation: An Unexpected Observation

Abstract. *The study of homing movements of displaced newts* (Taricha rivularis) *revealed unexpected features of the migratory behavior of amphibians. Newts leaving the breeding stream in the spring move not directly uphill but at an angle carrying them upstream. When they emerge after summer estivation this tendency is not evident in captures made during the autumn and winter. During the latter period, however, newly metamorphosed frogs* (Rana boylii) *show the same pronounced upstream migration that characterizes* T. rivularis *in the spring.*

The phenomenon we describe was encountered in experiments designed to determine whether displaced newts initiate their homing journey by oriented migration or random search. For this purpose "land traps" (wire-mesh fences that funnel migrating animals into escape-proof cages) were installed at moderate distances (about 13.5 to 215 m) from the banks of Pepperwood Creek, the stream in northwestern Sonoma County where most of our homing studies have been made (*1–6*). Several of the traps were constructed on the principle (Fig. 1) permitting one to determine whether, at the time of capture, the displaced animals were moving in an upstream or downstream direction. The design and results of these experiments have been described already (*1*); we now describe an odd and unexpected feature of the capture in these traps of nondisplaced members of the native amphibian population.

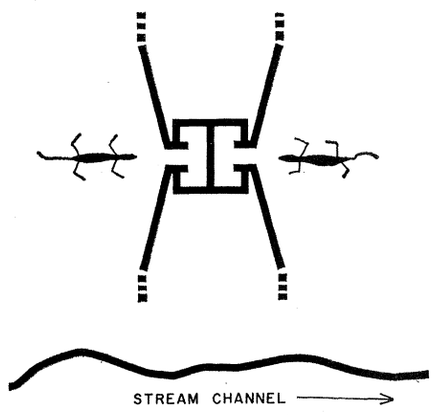

Fig. 1. Representation of traps installed near the streamside for determining whether migrating newts were moving in an upstream or downstream direction at the time of interception.

The traps were installed in autumn 1963, and the ensuing spring, as adults of the newt *Taricha rivularis* began to leave the stream late in the breeding season, we were surprised to find that most of the animals captured were present in the downstream halves of the cages. This showed that, at the time of interception, the newts were moving not directly uphill but at an angle carrying them upstream as well. For the remainder of that spring, and during the following years, we have been careful to record separately the numbers of captures in the upstream and downstream halves of all traps, not only of *T. rivularis*, but also of the frog *Rana boylii* and plethodontid salamanders *Aneides* and *Ensatina.*

The captures of *T. rivularis* conform to a well-defined seasonal pattern that is repeated each year (Fig. 2a). During the spring, especially in the later part of the breeding season when exodus from the water is already beginning, most captures consist of animals moving in the upstream direction. Captures cease during the ensuing dry months, when *T. rivularis* is in underground retreats, and resume with the onset of autumn rains. From then until the spring breeding season, a period during which *T. rivularis* forages on the forest floor when humidity and temperature permit, captures are distributed approximately equally in the two halves of the cages. One might perhaps expect a downstream orientation of migration during autumn and winter to compensate for the upstream movement in the spring, but, as seen in Fig. 2a, our captures reveal no evidence of this.

A possible interpretation previously mentioned (*1*) is that the upstream component of the terrestrial migration at the close of the breeding season is somehow an extrapolation of orientation to the current before leaving the water. While in the stream the animals characteristically face into the current, often working upstream against it; con-