are from cortical areas considered phylogenetically the most recent.

The ventromedial portions of the temporal lobe are thought to contain structures vital for the higher-order functions of memory and the acquisition of new learning in man (*1*, *21*). The striking memory deficits observed after damage to these regions of the brain are, perhaps, more understandable because the sensory information converging into these regions appears to be highly refined.

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References and Notes

1. W. B. Scoville and B. Milner, *J. Neurol. Neurosurg. Psychiat.* **20**, 11 (1957).
2. P. D. MacLean, *Psychosom. Med.* **11**, 338 (1949).
3. W. J. H. Nauta, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), pp. 5–28.
4. S. Ramón y Cajal, *Studies on the Cerebral Cortex*, L. L. Kraft, Transl. (Yearbook, London, 1955).
5. T. W. Blackstad, *Acta Anat.* **35**, 395 (1961).
6. G. von Bonin and P. Bailey, *The Neocortex of Macaca mulatta* (Univ. of Illinois Press, Urbana, 1947), pp. 1–101.
7. K. Brodmann, *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund der Zellenbaues* (Barth. Leipzig, 1909). Prepiriform cortex is used here in a general sense and includes both olfactory and periamygdaloid cortices. The ablations reported here mainly damaged the latter.
8. E. A. Walker, *J. Comp. Neurol.* **73**, 59 (1940).
9. W. J. H. Nauta, in *New Research Techniques of Neuroanatomy*, W. F. Windle, Ed. (Thomas Springfield, Ill., 1957), pp. 17–26.
10. R. P. Fink and L. Heimer, *Brain Res.* **4**, 369 (1967).
11. H. Stephan and O. J. Andy, in *The Primate Brain*, C. R. Noback and W. Montagna, Eds. (Appleton-Century-Crofts, New York, 1970), pp. 109–135.
12. G. W. Van Hoesen and D. N. Pandya, *Anat. Rec.* **169**, 445 (1971); W. R. Adey and M. Meyer, *Brain* **75**, 358 (1952).
13. In two recent reports (*14*, *15*), evidence is presented that a transitional or perirhinal region may surround the entorhinal cortex. Although we have not attempted to isolate this perirhinal region from conventional entorhinal areas in this report, an analysis of our results in light of these reports suggests that in terms of prepiriform, orbitofrontal, and TH projections, considerable overlap occurs. In agreement with Jones and Powell (*14*) however, the perirhinal region is easily set apart from entorhinal cortex after TE, TF, and TG ablations and corresponds to what we have called the lateral regions of areas 28a and 28b.
14. E. G. Jones and T. P. S. Powell, *Brain* **93**, 793 (1970).
15. J. L. Price and T. P. S. Powell, *J. Anat.* **110**, 105 (1971).
16. A Hjorth-Simonsen and B. Jeune, *J. Comp. Neurol.* **144**, 215 (1972).
17. D. N. Pandya and H. G. J. M. Kuypers, *Brain Res.* **13**, 13 (1967).
18. E. Stellar, *Psychol. Rev.* **61**, 5 (1954); N. Geschwind, *Brain* **88**, 237 (1965).
19. Although this report is concerned with sensory-limbic connections from the cortex, two reports dealing with sensory-limbic connections from subcortical structures are pertinent. P. D. MacLean and G. Creswell, *J. Comp. Neurol.* **138**, 265 (1970); R. Y. Moore, F. Karapas, N. J. Lenn, *Anat. Rec.* **169**, 382 (1971).
20. D. N. Pandya and V. B. Domesick, unpublished observations.
21. T. McLardy, *Int. J. Neurosci.* **1**, 113 (1970).
22. Supported by NIH grants NS-02378, -06209, and -10211. We thank N. Gershwind and T. McLardy for their interest and inspiration; V. DaForno, D. Hall, and F. Small for technical assistance; and A. Hjorth-Simonsen for sending preprints of his research and reprints of T. Blackstad's research on the hippocampal complex.

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Transfer Factor from Guinea Pigs Sensitive to Dinitrochlorobenzene: Absence of Superantigen Properties

Abstract. *Transfer factor from guinea pigs sensitive to dinitrochlorobenzene was not bound to an immunoadsorbent column that is specific for the dinitrophenyl determinant. The absence of the dinitrophenyl determinant on transfer factor suggested that the factor does not function as superantigen. The duration of the adoptive sensitivity, the small molecular weight, and the polypeptide or polynucleotide (or a combination) composition of the transfer factor are consistent with a derepressor function of the molecule.*

Early attempts to transfer delayed hypersensitivity with disrupted leukocytes suggested that a subcellular factor was involved (*1*). Since then, the passive transfer of delayed reactivity in man and in nonhuman primates with subcellular material (transfer factor) has been well established (*2*). Although transfers of delayed sensitivity with cell-free material in rodents have been inconsistent, methods have been described (*3*, *4*) for the collection, isolation, and testing of transfer factor from guinea pigs and rabbits sensitive to 2,4-dinitrochlorobenzene (DNCB). Essential steps for the collection of transfer factor from guinea pigs include (i) high donor-to-recipient ratios (at least 6 : 1); (ii) rapid collection of sensitive cells without their being washed; and (iii) incubation of cells at high concentrations [10^9 cells per 10 ml of Hanks balanced salt solution (HBSS)] at 37°C, without addition of serum or antigen. When these procedures are followed, incubation fluids, collected after 4 hours, were effective in transferring to other animals the contact sensitivity to DNCB (*4*).

Transfer factor has been described as a heat-sensitive, dialyzable, polypeptide or polynucleotide (or a combination) that is insensitive to deoxyribonuclease, ribonuclease, and trypsin (*5*, *6*). Accordingly, it has been suggested (*2*) that the specificity of transfer factor is imparted by either a superantigen or derepressor action. If transfer factor served as an immunogen, then transfer factor from animals sensitive to DNCB would carry the dinitrophenyl (DNP) determinant. Our studies (i) demonstrate that transfer factor from guinea pigs sensitized to DNCB does not bind to an immunoadsorbent that possesses covalently linked antibody to DNP, and (ii) describe additional physiochemical properties of transfer factor from guinea pigs. The results are interpreted as being consistent with a derepressor, rather than a superantigen, function for transfer factor.

Hartley guinea pigs were sensitized by six daily applications of 2 percent DNCB applied topically to a clipped area of the neck (*1*). Fourteen days after their initial treatment, the animals were skin tested with 1 percent DNCB, and were given 15 ml of mineral oil intra-abdominally. Lymph node and peritoneal exudative cells were collected (*3*, *4*) 48 hours later. Both the pooled lymph node cells and the pooled peritoneal exudative cells (each from 12 to 16 donors) were incubated separately for 4 hours in HBSS at 37°C, without addition of antigen (*4*). The incubation fluids were dialyzed for 18 hours, against 20 volumes of 0.9 percent saline, at 4°C. The dialyzate was concentrated by lyophilization and was applied to a Sephadex G-75 column (2.5 × 100 cm) equilibrated with 0.1*M* tris(hydroxymethyl)aminomethane (tris), *p*H 8; the effluent was monitored at an absorbance of 260 nm. Transfer factor was associated with a fraction eluted about 350 ml beyond the void volume of the column. Transfer factor activity was evaluated by contact skin tests of 1 percent DNCB in olive oil, which were applied

48 hours after intraperitoneal injection of the isolated fractions (*3, 4*). Control skin tests, with 1 percent citraconic anhydride, with 15 percent *o*-chlorobenzoyl chloride, and with olive oil, were performed on all transfer recipients and on untreated animals; the tests were negative.

Half of the transfer factor preparation was tested for activity by injecting it into guinea pigs and then performing skin tests. The remaining material was applied to an immunoadsorbent that binds DNP [prepared by chemical conjugation of rabbit antibody to DNP with Sephadex G-25 activated by cyanogen bromide (*7*)]. Transfer factor preparations applied to the immunoadsorbent specific for DNP were collected as two fractions; the unbound material that passed through the column (1 × 25 cm) with 300 ml of 0.1*M* tris, *p*H 8, and the material eluted in 6*M* urea (*8*). Transfer factor, from animals sensitive to DNCB, was not bound by the immunoadsorbent specific for DNP (Table 1) inasmuch as the fractions eluted with 6*M* urea did not elicit contact sensitivity in any of the recipients of it. The comparable activity observed in untreated transfer factor preparations and in the unbound fractions indicated that biological activity was not adsorbed by the immunoadsorbent specific for DNP and, as far as could be determined, this binding characteristic of the immunoadsorbent was not significantly altered at the completion of the elutions (*7*).

Sequential transfer experiments in man have suggested that passive sensitization is not initiated by antigen transfer (*2*). In addition, the presence of antigen in transfer preparations could not be demonstrated by lymphocyte transformation (*9*) or equilibrium dialysis (*6*). In the DNCB system, attempts to locate DNP groups on transfer factor preparations by inhibition of activity with antibody to DNP were also unsuccessful (*10*). These experimental results would be consistent with the idea that transfer factor does not act as immunogen; however, they do not exclude the possibility of small amounts of antigen in these preparations. The DNP groups that are not detectable by other means can still be bound by the immunoadsorbent specific for DNP. Thus, we have attempted to eliminate the possibility of passive sensitization by transfer factor that contains an antigenic determinant in minute concentrations. Although it is possible that the DNP determinant could be sterically restricted from the binding character of the adsorbent, the small size of the molecule makes this very unlikely. In addition, the immunoadsorbent effectively bound compounds of DNP (DNP_{10}-bovine serum albumin, DNP-glucagon, and DNP-lysine) with a range of molecular weights that would include that of transfer factor.

Table 1. The passive transfer of sensitivity to DNCB in guinea pigs with transfer factor from a immunoadsorbent specific for DNP. Skin tests on recipients were performed with 1 percent DNCB in olive oil, 48 hours after intraperitoneal injection of transfer factor. The results were graded 24 hours later, as follows: 0, no detectable reaction; 1, patchy erythema; 2, homogeneous erythema; 3, homogeneous erythema and induration; 4, homogeneous erythema, induration, and raised reaction site.

Experiment	Cell source*	Transfer factor activity of		
		Untreated transfer factor	Immunoadsorbent isolates	
			Unbound	Bound, eluted
1	PEC	3	3	0
	LNC	2	2	0
2	PEC	4	3	0
	LNC	2	2	0
3	PEC	3	3	0
	LNC	1	1	0
4	PEC	3	4	0
	LNC	3	3	0

* Transfer factor isolated from PEC (peritoneal exudative cells) and LNC (lymph node cells).

The duration of adoptive immunity to DNCB was determined by skin testing transfer recipients at monthly intervals after they had experienced transfer factor stimulation. In all cases, the recipient animals received their first, and only, skin test on the assay day. Of 16 recipients of transfer factor 15 initially demonstrated activity when skin tested 48 hours after intraperitoneal inoculation of transfer factor. Animals receiving skin tests at 1 month after injection of transfer factor continued to demonstrate contact sensitivity (six out of eight animals). We observed that the ability to respond to a 1 percent DNCB skin test, at 3 and 6 months after transfer factor challenge, was reduced to about 50 percent (four out of eight animals).

Measurements of the duration of adoptive immunity in man indicate that passive sensitization with transfer factor may last for years (*2*). Our results demonstrated that a passive sensitivity to DNCB can last for at least 6 months in some animals, without applying repeated skin tests, in a system free from spontaneous conversion to a sensitized state.

The molecular weight of transfer factor was estimated by two independent methods. Synthetic boundary ultracentrifugation (*11*) indicated that the isolated transfer factor has a sedimentation constant ($s_{20,w}$) of 0.2 to 0.3. In a second experiment, a Sephadex G-75 column (2.5 × 100 cm) was calibrated with carbohydrate-free compounds of known molecular weight (*12*). The elution profile of transfer factor was determined and its molecular weight was estimated by comparison with the standardized elution curve (*13*). Both methods indicated that the molecular weight of transfer factor is between 1000 and 2000.

Our initial chemical analysis of the isolated transfer factor both confirms and extends the previous descriptions of this material (*4*) that suggested a polypeptide-polynucleotide composition. Our preparations were orcinol- and biuret-positive; the ratio of absorbance at 260 and 280 nm was 1.7. After the transfer factor was hydrolyzed by acid, it was subjected to thin-layer chromatography for nucleotides which indicated the presence of adenine, guanine, and uracil in the preparation (*14*). We found, by amino acid analysis (*15*), most of the naturally occurring amino acids except those containing sulfur (methionine and half-cystine); valine, serine, and glycine were present in greater concentrations than were the other amino acids.

That transfer factor does not function as a superantigen is suggested by: (i) its biochemical and physical properties; (ii) the absence of antigen transfer; and (iii) the failure of the isolated transfer factor from guinea pigs sensitized to DNCB to be bound to an immunoadsorbent that binds DNP. The duration of the adoptive immunity, together with the properties mentioned above, are consistent with the transfer factor having a derepressor function. However, this remains to be determined by direct experiments.

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References and Notes

1. W. S. Jeter, M. M. Tremaine, P. M. Seebohm, *Proc. Soc. Exp. Biol. Med.* **86**, 251 (1954); H. S. Lawrence, *J. Clin. Invest.* **33**, 951 (1954); *ibid.* **34**, 219 (1955).
2. H. S. Lawrence, *Advan. Immunol.* **11**, 196 (1969).
3. R. K. Guthrie, W. S. Ellis, C. H. Brock, *Proc. Soc. Exp. Biol. Med.* **126**, 1 (1967); R. K. Guthrie and W. J. Nunez, *Amer. Rev. Resp. Dis.* **102**, 398 (1970); D. R. Burger and W. S. Jeter, *Bacteriol. Proc.* **69**, 7 (1969); D. R. Burger, W. S. Cozine, D. J. Hinrichs, *Proc. Soc. Exp. Biol. Med.* **136**, 1385 (1971); A. Ahmed and W. S. Jeter, *Bacteriol. Proc.* **71**, 241 (1971).
4. D. R. Burger and W. S. Jeter, *Infect. Immun.* **4**, 575 (1971).
5. H. S. Lawrence, S. Al-Askari, J. R. David, E. C. Franklin, B. Zweiman, *Trans. Ass. Amer. Physicians* **76**, 84 (1963); H. S. Lawrence and B. Zweiman, *ibid.* **81**, 240 (1968); P. Baram, L. Yuan, M. M. Mosko, *J. Immunol.* **97**, 407 (1966); M. P. Arala-Chaves, E. G. Lebacq, J. F. Hereman, *Int. Arch. Allergy Appl. Immunol.* **31**, 353 (1967); P. Fireman, M. Boesman, Z. H. Haddad, D. Gitlin, *Science* **155**, 337 (1967); M. W. Brandris *J. Clin. Invest.* **47**, 2152 (1968); D. R. Burger and W. S. Jeter, *Bacteriol. Proc.* **69**, 6 (1969).
6. P. Baram and M. M. Mosko, *Immunology* **8**, 641 (1965).
7. Rabbit antibody to DNP was isolated by elution of 7*S* globulins with high affinity in 6*M* urea from a trinitrophenyl (TNP)-cellex immunoadsorbent. The TNP-cellex immunoadsorbent was prepared by conjugating 10 g of cellex AE with 0.5 g of trinitrobenzene sulfonic acid in 50 ml of 0.3*M* cacodylate buffer, *p*H 6.8, at room temperature for 60 minutes. The resin was washed, in sequence, with 5 volumes of cacodylate saline, 5 volumes of water, 5 volumes of 0.2*M* glycine-HCl, 10 volumes of water, 5 volumes of 0.1*M* bicarbonate, and 10 volumes of water; the cycle was repeated 20 times. The resin was poured into a column, was washed with 15 volumes of 8*M* urea four times, and was equilibrated in 0.1*M* cacodylate saline, *p*H 6.8. Recovery of antibody to DNP, on three consecutive runs, was 50, 70 and 80 percent. Of the total isolated antibody to DNP, 50 mg was conjugated to Sephadex G-25 activated with cyanogen bromide, according to: P. Cuatrecasas, M. Welcheck, C. B. Anfinsen, *Proc. Nat. Acad. Sci. U.S.* **61**, 636 (1968); A. Malley, L. Baecher, B. Willson, F. Perlman, *J. Allergy* **47**, 131 (1971). The immunoadsorbent with specificity for DNP was saturated by 12 mg of DNP_{10}-BSA, 1.4 mg of DNP-glucagon, or 0.09 mg of DNP-lysine eluted in 6*M* urea. These values were calculated by microkjeldahl determinations on pooled elution runs. Equivalent values were recorded after eight elutions of the isolated transfer factor.
8. Transfer factor activity was not diminished by repeated treatment with 0.1*M* tris buffer, *p*H 8, or with 6*M* urea.
9. D. E. Thor and S. Dray, *J. Immunol.* **101**, 469 (1968); F. T. Valentine and H. S. Lawrence, *Science* **165**, 1014 (1969).
10. D. R. Burger and W. S. Jeter, unpublished data.
11. Ultracentrifugation performed in model E ultracentrifuge (Beckman), at 20°C, at 59,780 rev/min for 160 minutes.
12. Molecular weights as follows: glucagon, 3,500; cytochrome c, 12,400; ribonuclease, 13,700; chromotrypsinogen, 25,000; ovalbumin, 45,000; bovine serum albumin, 67,000.
13. T. C. Laurent and J. Killander, *J. Chromotogr.* **14**, 317 (1964); P. R. Carnegie, *Biochem. J.* **95**, 90 (1965).
14. F. C. Haer, *An Introduction to Chromatography* (Ann Arbor Science Publisher, Ann Arbor, Mich., 1970), pp. 143–151.
15. D. A. Spackman, *Fed. Proc.* **22**, 244 (1963).

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Active Transport of Potassium and Chloride in an Identifiable Molluscan Neuron

Abstract. *Direct measurements of intracellular K^+ and Cl^- activities before and after blockage of cellular metabolic processes indicate that K^+ is actively transported inwardly and Cl^- is actively transported outwardly from the giant cell of the abdominal ganglion of* Aplysia. *The rewarming of cells that have been cooled to 1° ± 1°C causes K^+ to be taken up and Cl^- to be extruded against electrochemical gradients.*

The active transport of K^+ and Cl^- has been convincingly demonstrated in only one neural structure, the squid axon (*1*, *2*). In other nervous tissue, the bulk of the evidence for active transport of either ion has been indirect (*3*) or incomplete (*4*). We have directly measured intracellular K^+ activity (a^i_K), intracellular Cl^- activity (a^i_{Cl}), and membrane resting potential (E_M) continuously, and often simultaneously, in the giant neuron R2 (*5*) of the *Aplysia* abdominal ganglion. Control measurements were made over as many as 4 to 6 hours; measurements were also made after exposure to either cooling, ouabain, cyanide, or 2,4-dinitrophenol. From the results, we conclude that (i) K^+ is actively transported into this neuron, (ii) Cl^- is actively transported out of this neuron, and (iii) the transport systems for K^+ and Cl^- are different.

We recorded a^i_K and a^i_{Cl} using liquid ion-exchanger microelectrodes (*6*), calibrated prior to and after the measurement of intracellular activities with KCl solutions varying in activity from $6.0 \times 10^{-1}M$ to $9 \times 10^{-3}M$. We measured membrane potentials using glass micropipettes filled with 3*M* potassium chloride (KCl), 2.0*M* sodium citrate ($Na_3C_6H_5O_7$), or 0.6*M* sodium sulfate (Na_2SO_4) with no difference in results. We found it easier to impale the cell with these electrodes after we removed the connective tissue capsule and then washed the preparation for 30 to 60 minutes with artificial seawater (*7*). After impalement, continuous simultaneous measurements of a^i_K, a^i_{Cl}, and E_M were made in 23 cells until the values became stable (40 to 80 minutes). At this time, the equilibrium potentials for Cl^- and K^+, (E_{Cl}, E_K), and E_M were -55 ± 1.0, -80 ± 0.7, and -50 ± 1.0 mv (mean ± standard error of the mean), respectively. Both E_K and E_{Cl} differ significantly from E_M ($P < .01$). When the control measurements were extended for another 300 minutes (seven cells), E_{Cl}, E_K, and E_M showed little change, having values of -56 ± 2.0, -81 ± 0.9, and -50 ± 2.3 mv, respectively. The giant cells were normally quiescent, maintaining an E_M of about −50 mv and an input membrane resistance (R_T) of about 1.0 megohm and, after stimulation, action potential overshoots of +30 to +50 mv. It seemed reasonable then to assume that the cells were in a steady state within 40 to 80 minutes after impalement (*8*). Since E_K, E_{Cl}, and E_M differ

Table 1. Effects of cooling and treatment with ouabain on K^+ and Cl^- activities in the *Aplysia* giant cell. Values are the mean ± the standard error of the mean; the numbers in parentheses refer to the number of cells; a^i_K and a^i_{Cl} were measured concurrently in five cooled cells and six ouabain-treated cells. All control data refer to steady-state values obtained just before the beginning of treatment. The 70-minute post-treatment time was chosen as the time when $E_{Cl} = E_M$ after cooling to 1°C, the ouabain data being given at the same time for purposes of comparison.

Cell	a^i_K (m*M*)	E_K (mv)	a^i_{Cl} (m*M*)	E_{Cl} (mv)	E_M (mv)
Control	187 ± 7.0 (7)	−83 ± 1.0 (7)	34 ± 1.8 (11)	−59 ± 1.2 (11)	−49 ± 1.6 (11)
70 minutes after cooling	149 ± 4.5 (7)	−78 ± 0.6 (7)	44 ± 2.6 (11)	−48 ± 1.2 (11)	−48 ± 1.4 (11)
Control	165 ± 6.0 (16)	−79 ± 0.9 (16)	36 ± 1.7 (6)	−56 ± 1.7 (6)	−46 ± 1.8 (16)
70 minutes after exposure to ouabain	127 ± 5.4 (16)	−73 ± 1.0 (16)	40 ± 1.1 (6)	−53 ± 1.3 (6)	−37 ± 1.5 (16)