Crystals of CT from the above studies were washed and redissolved, and their activity was determined in two separate bioassays. Dose-dependent activation of rabbit ileal epithelial cell adenylate cyclase (*21*) and dose-dependent induction of mouse adrenal cell morphological changes (*22*) were compared with the same phenomena induced by CT from the NIH Cholera Advisory Committee and that commercially available from Schwarz/Mann. In the mouse adrenal cell system, duplicate bioassay of dissolved crystals and separated mother liquor, in parallel with CT which had not been manipulated, showed no significant difference in specific activity. In this assay, the transition to observable cell rounding occurred between 2 and 4 pg per 250 μl per well with each of the above CT samples. Complete dose-response determinations of the activation of rabbit ileal epithelial cell adenylate cyclase by dissolved crystalline material yielded a dissociation constant (K_d) of $5 \times 10^{-10} M$, in comparison with $K_d = 5 \times 10^{-9} M$ (*21*) for preparations from NIH and Schwarz/Mann. Both values are in agreement with values reported by Cuatrecasas (*23*) for the interaction of CT with fat cell ($K_d = 4.6 \times 10^{-10} M$) and liver ($K_d = 1.1 \times 10^{-9} M$) membrane.

In conclusion, (i) the density of the crystal is consistent with five, rather than six, B subunits, and (ii) there is no expression of a molecular rotation axis in the symmetry of the crystal lattice. This, of course, does not rule out rotational symmetry in the arrangement of the B subunits which could exist within the asymmetric unit.

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References and Notes

1. R. A. Finkelstein, *Crit. Rev. Microbiol.* **2**, 553 (1973).
2. M. Field, *N. Engl. J. Med.* **284**, 1137 (1971).
3. P. Cuatrecasas, *Biochemistry* **12**, 3558 (1973); J. Holmgren, I. Lönnroth, L. Svennerholm, *Infect. Immun.* **8**, 208 (1973); C. A. King and W. R. van Heyningen, *J. Infect. Dis.* **127**, 639 (1973).
4. M. Field, G. R. Plotkin, W. Silen, *Nature (London)* **217**, 469 (1968); M. Field, D. Fromm, C. K. Wallace, W. B. Greenough, III, *J. Clin. Invest.* **48**, 24a (abstr.) (1969); M. Field, D. Fromm, Q. Al-Awqati, W. B. Greenough, III, *ibid.* **51**, 796 (1972).
5. R. M. Friedman and L. D. Kohn, *Biochem. Biophys. Res. Commun.* **70**, 1078 (1976).
6. H. D. Kiefer and H. S. Kantor, in *Cyclic Nucleotides and Regulation of Cell Growth*, M. Abou-Sabe, Ed. (Dowden, Hutchinson and Ross, Stroudsburg, Pa., in press).
7. F. D. Ledley, B. R. Mullen, G. Lee, S. M. Aloj, P. H. Fishman, L. T. Hunt, M. O. Dayhoff, *Biochem. Biophys. Res. Commun.* **69**, 852 (1976); B. R. Mullen, P. H. Fishman, G. Lee, S. M. Aloj, F. D. Ledley, R. J. Winand, L. D. Kohn, R. O. Brady, *Proc Natl. Acad. Sci. U.S.A.* **73**, 842 (1976).
8. A. Kurosky, D. E. Markel, J. W. Peterson, W. M. Fitch, *Science* **195**, 299 (1977).
9. R. A. Finkelstein, M. Boesman, S. H. Neoh, M. K. LaRue, R. Delaney, *J. Immunol.* **113**, 145 (1974); D. G. Klapper, R. A. Finkelstein, J. D. Capra, *Immunochemistry* **13**, 605 (1976); J. Sattler, H. Weigandt, J. Staerk, T. Kranz, H. J. Ronneberger, R. Schmidtberger, H. Zilg, *Eur. J. Biochem.* **57**, 309 (1975); S. van Heyningen, *J. Infect. Dis.* **133** (Suppl.), S5 (1976); A. Kurosky, D. E. Markel, B. Touchstone, J. W. Peterson, *ibid.*, p. S14; C. Y. Lai, E. Mendez, D. Chang, *ibid.*, p. S23; O. Ohtomo, T. Muraoka, A. Tashiro, Y. Zinnaka, K. Amako, *ibid.*, p. S31.
10. D. M. Gill, *J. Infect. Dis.* **133** (Suppl.), S55 (1976). M. A. Wheeler, R. A. Solomon, C. Cooper, L. Hertzberg, H. Mehta, N. Miki, N. W. Bitensky, *ibid.*, p. S89; C. A. King, W. E. van Heyningen, M. Gascoyne, *ibid.*, p S75; F. Berkenbile and R. Delaney, *ibid.*, p. S82.
11. J. Holmgren and I. Lönnroth, *ibid.*, p. S64.
12. D. M. Gill, *Biochemistry* **15**, 1242 (1976); Y. Nakashuna, P. Napiorkowski, D. E. Schafer, W. H. Konigsberg, *FEBS Lett.* **68**, 275 (1976); C. Y. Lai, E. Mendez, D. Chang, M. Wang, *Biochem. Biophys. Res. Commum.* **74**, 215 (1977).
13. R. A. Finkelstein and J. J. LoSpalluto, *J. Exp. Med.* **130**, 185 (1969).
14. J. W. Peterson, J. J. LoSpalluto, R. A. Finkelstein, *J. Infect. Dis.* **126**, 617 (1972); N. F. Pierce, *J. Exp. Med.* **137**, 1009 (1973); P. Cuatrecasas, *Biochemistry* **12**, 3577 (1973).
15. R. A. Finkelstein and J. J. LoSpalluto, *Science* **175**, 529 (1972).
16. ———, *J. Infect. Dis.* **121** (Suppl.), S63 (1970); R. A. Finkelstein, K. Fujita, J. J. LoSpalluto, *J. Immunol.* **107**, 1043 (1971).
17. A. Hampel, M. Labanauskas, P. G. Connors, L. Kirkegard, U. L. RajBhandary, P. B. Sigler, R. M. Bock, *Science* **162**, 1384 (1968); A. McPherson, *Methods Biochem. Anal.* **23**, 249 (1976).
18. A. McPherson, *J. Biol. Chem.* **251**, 6300 (1976).
19. E. Westbrook, *J. Mol. Biol.* **103**, 659 (1976).
20. J. J. LoSpalluto and R. A. Finkelstein, *Biochim. Biophys. Acta* **257**, 158 (1972).
21. H. C. Kiefer *et al.*, *Biochem. Biophys. Res. Commun.* **66**, 1017 (1975).
22. S. T. Donta, M. King, K. Sloper, *Nature (London) New Biol.* **243**, 246 (1973).
23. P. Cuatrecasas, *Biochemistry* **12**, 3547 (1973).
24. J. Holmgren, J.-E. Månsson, L. Svennerholm, *Med. Biol.* **52**, 229 (1974).
25. We thank E. Westbrook for help with the density measurements. Supported by PHS grants GM 22324 and GM 15225 and NSF grant PCM74-15075 (to P.B.S.); PHS grants GM23127 and RR 05370 (to H.C.K); and PHS research grant AI-08877 (to R.A.F.) under the U.S.–Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases.

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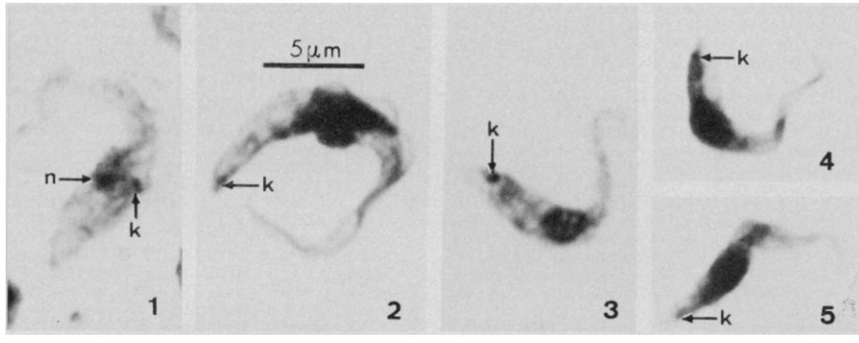
Infectivity Reacquisition by *Trypanosoma brucei brucei* Cultivated with Tsetse Salivary Glands

Abstract. *Reacquisition of infectivity for mice was observed in cultures of* Trypanosoma brucei brucei *grown in the presence of tsetse fly salivary gland explants in a medium based on the amino acid composition of* Glossina *hemolymph and containing fetal bovine serum. High infection rates were obtained in mice inoculated with about 1.5 × 10⁸ organisms. Infectivity reacquisition was correlated with invasion of the salivary glands by the parasites. Few small trypanosomes with subterminal kinetoplasts (metacyclic-like forms) were found in the infective inoculums. The parasitemias in mice consisted of pleomorphic cultivable trypanosomes. Cultures initiated by these organisms and then placed with the head–salivary gland preparations became infective for mice.*

Trypanosoma brucei brucei, an important cause of disease in cattle, constitutes a prime factor in human malnutrition in Africa. Other subspecies of *T. brucei*, *T. b. rhodesiense* and *T. b. gambiense*, cause human sleeping sickness. Heretofore, infectivity for mammalian hosts was lost, typically irreversibly, by subspecies of *T. brucei* after they were established in culture. The noninfective culture forms resemble in their structure (*1*) and antigenic composition (*2*) the stages of the parasites found in the alimentary tract of their natural vectors, tsetse flies. Further development culminating in the formation of metacyclic trypanosomes infective for mammals occurs in the salivary glands of some of the flies, but induction of this development in cultures could not be controlled. In the present report we describe a method whereby infectivity reacquisition can be controlled in *T. b. brucei* cultures. Our results can be viewed as a first step toward defining the factors that control the completion of the parasite's life cycle to the infective (metacyclic) stage. The system we describe might also lead to development of a vaccine against trypanosomiasis.

Improved methods for the cultivation of arthropod tissues and organs have facilitated studies of the vector stages of various parasites. Records of attempts to employ tsetse organ cultures to produce infective stages of salivarian trypanosomes were published by Trager (*3*) and Nicoli and Vattier (*4*). Using this system with *Trypanosoma vivax*, Trager succeeded in infecting two sheep, but neither he nor Nicoli and Vattier were able to infect rats with *T. b. brucei* (*3*) or *T. b.*



Figs. 1 to 5. Photomicrographs of Giemsa-stained *T. b. brucei* found in infective inoculums derived from cultures grown in the presence of *G. m. morsitans* head–salivary gland explants. The scale in Fig. 2 pertains to all five figures; final magnification in print is about ×2770. Fig. 1. Epimastigote with the kinetoplast (*k*) at the level of the nucleus (*n*), a situation often found in epimastigotes (although somewhat distorted, this organism is shown because it was the most typical epimastigote stage seen in the preparations examined). Fig. 2. Large trypanosome with a nearly terminal kinetoplast (*k*). Figs. 3 to 5. Metacyclic-like trypanosomes, with subterminal kinetoplasts (*k*).

rhodesiense (*4*) grown with *Glossina* organs. In other studies (*5*), negative results were obtained with regard to infectivity for mice of *T. b. brucei* grown for 5 to 20 days with tsetse organ cultures in modified Trager's medium (*6*). As a control for this method of cultivating salivarian trypanosomes with *Glossina* organ cultures, attempts were made (*5*) to grow *T. b. brucei* with mosquito (*Aedes aegypti*) organ cultures either in Trager's modified medium with mosquito pupal extract or in Schneider's (*7*) medium, both supplemented with 20 percent fetal bovine serum; the trypanosomes failed to multiply in these systems. No growth of *T. b. brucei* was observed in a system involving tick (*Rhipicephalus appendiculatus*) organs either in modified Trager's medium without pupal extract or in Varma-Pudney medium 4 (*8*) with 10 percent fetal bovine serum. Evidently, organs from nonvector arthropod species failed to support growth of the trypanosomes.

Recently, a medium for the cultivation of *Glossina* organs and trypanosomatids was described (*9*), which contains salt; sugar; an organic acid composition similar to that recommended by Grace (*10*), with amino acid constituents based on that of tsetse hemolymph (*11*); and 20 percent fetal bovine serum. This medium is capable of maintaining tsetse organs for prolonged periods. It also supports excellent growth of *T. b. brucei* culture forms, which morphologically and antigenically resemble the procyclic stages of this parasite found in the alimentary tract of the vector. (In the procyclics, the kinetoplast is located about midway between the nucleus and the posterior end of the cell.) The present research was undertaken to ascertain whether, by using the new medium for a *Glossina* salivary gland–trypanosome system, we could reproduce that part of the cycle which *T. b. brucei* completes in the vector and which culminates in the formation of the infective metacyclic stages (short flagellates with a subterminal kinetoplast).

Newly deposited *Glossina morsitans morsitans* pupae (Tsetse Research Laboratories, Bristol, England) were incubated in pots of sterile sand at 28°C and with a relative humidity of 65 to 80 percent.

Puparia containing flies about to emerge were surface-sterilized with White's solution (*3*) for 15 minutes and washed several times in insect balanced salt solution (IBSS) (*12*) containing 100 international units of penicillin, 100 μg of streptomycin, and 10 μg of Fungizone (for tissue culture; Squibb) per millilter. The washed puparia were dissected in IBSS. The heads with attached salivary glands were removed from the flies and placed either on 9 by 35 mm cover glasses in Leighton tubes (henceforth referred to as tubes) or in 25-cm² Falcon flasks (henceforth referred to as flasks) containing about 0.2 and 0.5 ml of culture medium, respectively. Five head–salivary gland explants were placed in the tubes and 13 to 24 explants in the flasks.

In all experiments, about 0.5 or 1.0 ml of the culture medium with 10^6 (per milliliter) completely transformed *T. b. brucei* procyclics were added to tubes and flasks containing the head–salivary gland preparations. The numbers of trypanosomes increased about 25-fold during 48 hours. The cultures were maintained by removing the parasite-containing medium and replacing it by an equal volume of fresh medium every 2 days.

The conditions of experiment 1 are described separately below. In experiments 2 to 4, the inoculums for mice were prepared as follows: (i) The trypanosome-containing supernatant fluids from cultures with and without obviously invaded salivary glands were pooled, then centrifuged at about 1100*g* for 10 minutes. (ii) The pellet was suspended in 0.4 to 0.5 ml of medium and inoculated into a mouse via subcutaneous and intraperitoneal routes, 0.2 to 0.25 ml by each route. Mice were injected every second day, or occasionally daily. The numbers of infections were calculated for periods during which the cultures were capable of producing parasitemias.

All experiments included control mice inoculated with trypanosomes grown in the absence of tsetse organs. In most instances, the numbers of the organisms in the control inoculums equaled those in the experimental inoculums (see Table 1); some of the former were much higher, up to 1.3×10^9 cells. Parasitemias did not develop in any of the control mice during the period of observation. The tail blood from experimental and control mice was examined every second day for 25 days.

In experiment 1, bloodstream trypanosomes from a stabilate derived from a tsetse-transmissible *T. b. brucei* strain TREU 1193 were established in culture as described previously (*3*). Procyclic stages maintained in the culture medium alone for periods ranging from 10 days to 7 weeks were added to tubes containing tsetse salivary glands. After 14 to 30 days, some of the salivary glands and proboscises became invaded by trypanosomes. Two or three infected glands were triturated in 0.4 ml of fresh medium, and the suspension was inoculated subcutaneously and intraperitoneally into mice. Trypanosome-containing supernatant fluids from some of the cultures with infected glands were also inoculated into mice. Two of the 15 mice injected with suspensions of triturated glands and one of the six animals inoculated with supernatant fluids from individual cultures became infected with trypanosomes.

Experiments 2 to 4 (see Table 1) indicated the following: (i) There is a definite relation between the time of infectivity reacquisition by the cultures and that at which the invasion of the salivary glands by the parasites was first noted. About 10 percent of the glands were invaded in the course of an experiment. It is possible, however, that because the salivary glands were at times concealed under the heads, the invasions might

Table 1. Infectivity reacquisition by *T. b. brucei* cultivated with *Glossina* salivary glands.

Experiment number	Days in culture		Organ cultures set over period (days)	Days before first gland invasion	Period of infectivity (days)†	Inoculum size		Ratio of infected mice to total number
	Without glands	With glands*				From many tubes or flasks	From up to five tubes or one flask	
2‡	50	20	30	17	30	$\sim 1.5 \times 10^8$	$\sim 6.0 \times 10^7$	19/23
								2/11
3§	14	11	4	12	35	$\sim 1.5 \times 10^8$	$\sim 6.0 \times 10^7$	5/9
								1/8
4‖	14	10¶	6	12	17	$\sim 1.5 \times 10^8$		8/11

*Before infectivity restoration. †Periods during which the cultures were infective for mice. ‡Cultures initiated from TREU 1193 strain. §Cultures initiated with bloodstream forms from one of the mice infected in experiment 2. ‖Trypanosomes from experiment 3 cultures exposed to fresh tsetse head–salivary gland explants. ¶There appeared to be a 10-day lag between the time of exposure of the trypanosomes to the tsetse organs and that at which they became infective for mice. For additional remarks, see text.

have started earlier than indicated in Table 1 and some subsequent invasions might have been overlooked. (ii) Significantly higher infection rates were achieved in mice receiving large inoculums (about 1.5×10^8 organisms) obtained by pooling the trypanosomes from many cultures than in those given smaller inoculums (about 6×10^7 organisms) from one flask or up to five tubes.

Experiment 4 differed from the preceding two in that it was designed to ascertain whether infectivity of the parasites for mice could be prolonged by transferring them to flasks with fresh head–salivary gland explants. Trypanosomes with infectivity potential obtained from cultures used for experiment 3 were added to four flasks containing the explants. As indicated in Table 1, 10 days elapsed before these parasites became infective for mice. It is possible, however, that this lag period was not real, because the inoculums used during the first 10 days after exposure of the trypanosomes to the fresh organ explants were relatively small, being derived from one (first inoculation) or two (two following inoculations) flasks.

Trypanosomes morphologically similar to those found in the midgut and proventriculus of an infected tsetse fly were the most abundant in Giemsa-stained smears obtained from the infective inoculums. There were, however, in these smears occasional epimastigote-like forms (Fig. 1) and also a few shorter organisms with subterminal kinetoplasts (Figs. 2 to 5); of these latter, many resembled morphologically the metacyclic stages (Figs. 3 to 5). Stained preparations of trypanosomes grown without tsetse organs (control cultures) contained only midgut- and proventricular-like organisms.

The prepatent periods in the infected mice ranged from 5 to 8 days and were followed by relapsing parasitemias. The course of the infections resembled that of parasitemias initiated in mice by forms from infected *Glossina* or of those caused by strains that underwent only relatively few mouse-to-mouse syringe passages after an initial infection by the vector. The "culture-transmitted" trypanosomes were pleomorphic and fully cultivable. Preliminary agglutination tests indicate that the predominant variant-specific antigens of these populations differ from that of the parent TREU 1193 strain.

Our salivary gland–trypanosome system in vitro reproduces to some extent the conditions prevailing in the vector. Infectivity reacquisition in cultures coincides with invasion of the salivary glands by the parasites. Many glands observed in the course of the experiments became invaded about 20 days after their exposure to the flagellates. This period corresponds approximately to that required for the completion of the *T. brucei* cycle in the tsetse fly. Smaller inoculums (about 6×10^7 organisms), containing triturated glands or derived from the supernatant fluids of the trypanosome-tsetse organ cultures, were less likely to give rise to parasitemias in mice than were large inoculums with about 1.5×10^8 organisms, derived from numerous cultures. These findings reflect the relative scarcity of infective forms in the cultures. They correlate well with the results of microscopic examinations, during which few organisms with a subterminal kinetoplast were noted in samples of infective material. These observations and the scarcity of the epimastigote forms in such samples suggest the existence of certain differences between our system and that represented by an infected *Glossina*. The altered conditions in the explanted salivary glands, which ceased to contract after about 9 days in culture, might be responsible for some of these differences.

Only the data of Trager (*3*) appear to be directly relevant to our work. He produced *T. vivax* parasitemias in two sheep by inoculating them with trypanosomes cultivated in the presence of tsetse organs in hanging drop cultures. In later work Trager (*13*) obtained cultures of *T. vivax* with large numbers of epimastigotes attached to the fly tissues (especially peritrophic membrane), but these cultures were not tested for infectivity. Although much information is available on the restoration of infectivity for mice to *T. b. brucei* and *T. b. rhodesiense* cultures grown in blood agar–base media with various additives (*14*), the results published on this subject are not readily explicable or entirely consistent. Moreover, in some instances the experimental parasitemias had unusually long prepatent periods, and many of them were unlike those commonly observed in rodent hosts.

We have developed a culture system capable of yielding reproducible results with regard to infectivity reacquisition by *T. b. brucei*. The applicability of this system to other salivarian species and subspecies should be investigated.

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References and Notes

1. R. F. Steiger, *Acta Trop.* **30**, 64 (1973).
2. B. M. Honigberg, I. Cunningham, H. A. Stanley, K.-E. Su-Lin, A. G. Luckins, *Exp. Parasitol.* **39**, 496 (1976).
3. W. Trager, *Ann. Trop. Med. Parasitol.* **53**, 473 (1959).
4. J. Nicoli and G. Vattier, *Bull. Soc. Pathol. Exot.* **57**, 213 (1964).
5. I. Cunningham, thesis, University of Edinburgh (1974).
6. ———, *Exp. Parasitol.* **33**, 34 (1973).
7. I. Schneider, *J. Exp. Zool.* **156**, 91 (1964).
8. M. G. R. Varma and M. Pudney, in *Proceedings of the Third International Colloquium on Invertebrate Tissue Culture*, J. Řeháček, D. Blaškovič, W. F. Hink, Eds. (Publishing House of

the Slovak Academy of Sciences, Bratislava, 1973), p. 135.
9. I. Cunningham, *J. Protozool.* **24**, 325 (1977).
10. T. D. C. Grace, *Nature (London)* **195**, 788 (1962).
11. I. Cunningham and J. S. Slater, *Acta Trop.* **31**, 83 (1974).
12. B. M. Jones and I. Cunningham, *Exp. Cell Res.* **23**, 386 (1961).
→ W. Trager, *J. Parasitol.* **61**, 3 (1975).
14. B. M. Honigberg, in *Chemical Zoology*, M. Florkin and B. T. Scheer, Eds., vol. 1, *Protozoa*, G. W. Kidder, Ed. (Academic Press, New York, 1967), p. (→ Y. Mendez and B. M. Honigberg, *J. Parasitol.* **58**, 1122 (1972).
15. This investigation was supported by research grants AI 00742-20, 21 from the National Institute of Allergy and Infectious Diseases.
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Sperm Autoantibodies in Vasectomized Rats of Different Inbred Strains

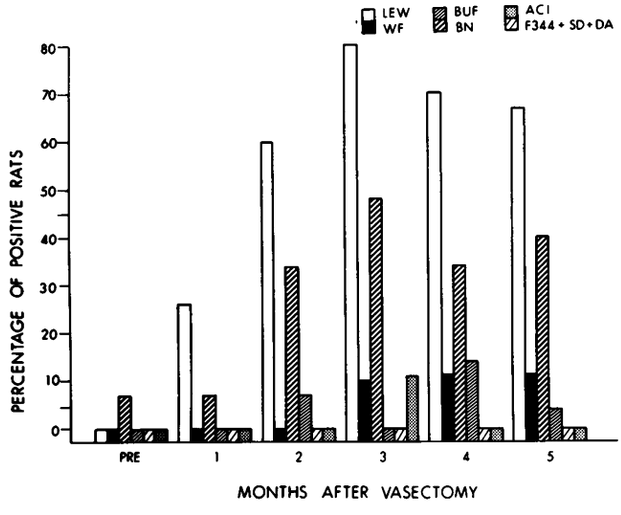
Abstract. *An immune response to antigens of spermatozoa occurs after vasectomy in rats of some inbred strains, but not in others. Antibodies to rat spermatozoa were detected by indirect immunofluorescence in some of the serums of vasectomized rats of the following strains: 80 percent of Lewis, 47 percent of Brown Norway, 13 percent of Buffalo, 12 percent of Wistar-Furth, and 11 percent of ACI rats. No such antibodies were detected in the serums of vasectomized Fischer, Dark Agouti, and Sprague-Dawley rats.*

Results of studies on the effects of vasectomy in rats are not uniform, some describing macroscopic or microscopic changes (or both) in the testes of vasectomized animals (*1*), others reporting no alterations (*2*). This disparity is not surprising, since these investigations were performed with different strains of outbred rats, and the results are therefore difficult to compare. Recent investigations have demonstrated that vasectomy may be followed by an autoimmune response to antigens of spermatozoa (*3*) and, in rabbits, by an immune complex-mediated orchitis (*4*). It has also been shown that rats, like mice and guinea pigs, possess histocompatibility-linked immune response genes (*5*) and that the appearance of some experimentally induced autoimmune diseases is under genetic control (*6–8*). For these reasons, we have investigated the production of antibodies to antigens of spermatozoa in bilaterally vasectomized rats of different strains, namely, inbred Lewis (LEW), Brown Norway (BN), Buffalo (BUF), ACI, Wistar-Furth (WF), Fischer (F344), Dark Agouti (DA), and outbred Sprague-Dawley rats (*9*).

A maximum of 80 percent of vasectomized LEW rats had circulating antibodies to rat spermatozoa, detectable by indirect immunofluorescence (IF) on sperm smears (*10*) (Fig. 1). Similar antibodies were also found in the serums of 47 percent of BN, 13 percent of BUF, 12 percent of WF, and 11 percent of ACI rats that had been vasectomized. In contrast, vasectomized F344, DA, and Sprague-Dawley rats did not have antibodies to sperm in their serums. The incidence of antibodies to spermatozoa in serums of vasectomized rats increased with time, reaching its maximum at 3 months after vasectomy in LEW, BN, and ACI and at 4 months in BUF and WF (Fig. 1).

Positive reactions obtained with LEW, BN, ACI, BUF, and WF serums were localized in the acrosomal region of rat spermatozoa (Fig. 2) and were obtained with sperm smears from LEW, BN, ACI, BUF, and WF as well as F344, DA, and Sprague-Dawley rats. This result indicates that acrosomal antigens involved in this immune response are present even in the spermatozoa of those rats like F344, DA, and Sprague-Dawley that did not produce antibodies to spermatozoa. The antibodies to sperm in the serums of positive animals were capable of reacting with spermatozoa from animals of the same inbred strain and also with spermatozoa from the positive animals themselves; thus they can be defined as autoantibodies.

Titers of antibodies to acrosomal antigens of spermatozoa ranged from 10 (or less than 10) to 320. The mean titers were 137 ± 39 in positive serums of LEW rats and 48 ± 30 in positive serums of BN rats. The few positive animals in the other strains had titers of 10 or less than 10. Serums from sham-vasectomized and untreated rats as well as serums from prevasectomy bleedings obtained from LEW, ACI, BUF, and WF rats did not contain antibodies to rat spermatozoa. The prevasectomy serum from one BN rat gave a positive reaction, with a titer



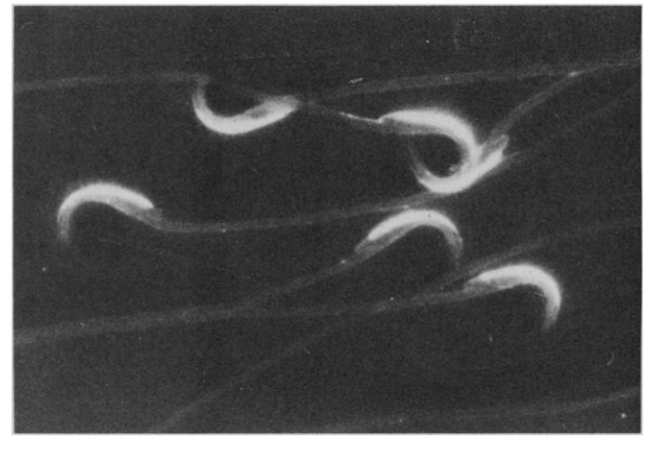


Fig. 1 (left). Incidence and time sequence of sperm antibody response in bilaterally vasectomized rats of different strains. Abbreviations: *LEW*, Lewis; *WF*, Wistar-Furth; *BUF*, Buffalo; *BN*, Brown Norway; *F344*, Fisher; *SD*, Sprague-Dawley; *DA*, Dark Agouti. Fig. 2 (right). Demonstration of antibodies to the acrosome of rat spermatozoa by indirect immunofluorescence (photographed at 100×).