LRF-induced release of LH. Furthermore, this inhibitory effect is evident in vitro at physiologic concentrations. When measured either by bioassay (*6*) or by radioimmunoassay (*7*), concentrations of melatonin in human serum attain values at night of approximately 1 n*M*, a dose which was shown in the present study to inhibit LH release. Other studies in our laboratory (*8*) have demonstrated that the in vitro response to melatonin is rapid, as evidenced by complete suppression of LRF-induced release of LH during a 90-minute incubation period. In addition, specificity of the response in organ culture is indicated by the finding (*8*) that two compounds closely related to melatonin, *N*-acetylserotonin and 5-methoxytryptamine, at concentrations as high at 100 n*M* do not affect the LH response to LRF.

In view of the finding that in the adult rat injection of melatonin into the third ventricle of the brain suppresses LH secretion, whereas injection into a hypophyseal portal vein has no effect on serum LH, Kamberi *et al.* (*9*) have suggested that melatonin inhibits the release of LRF into portal blood. Thus, it appears that melatonin may act both at the hypothalamic level and at the pituitary level to regulate LH secretion. However, their failure to observe suppression of serum LH by melatonin administered directly to the pituitary gland in vivo may be due to the low level of basal LH secretion. It may also be related to the age of the animals. Adult rats were used in their experiments, but neonatal rats were the source of the pituitary glands in our study.

The present finding that LRF stimulation of LH release can be modulated by physiological concentrations of a secretory product of the pineal gland may explain in part the known inhibitory effects of this gland on the reproductive system.

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References and Notes

1. R. J. Reiter, in *Handbook of Physiology: Endocrinology*, E. Knobil and W. H. Sawyer, Eds. (American Physiological Society, Washington, D.C., 1974), section 7, vol. 4, part 2, pp. 519–550; R. J. Wurtman, in *Textbook in Endocrinology*, R. H. Williams, Ed. (Saunders, Philadelphia, 1974), pp. 832–841.
2. J. E. Martin, L. Tyrey, J. W. Everett, R. E. Fellows, *Endocrinology* **94**, 556 (1974). A dose of 0.1 ng of this preparation of LRF stimulates a significant elevation in serum LH in the estrogen-progesterone–treated castrate female rat assay described by V. D. Ramirez and S. M. McCann [*ibid.* **73**, 193 (1963)].
3. J. E. Martin and D. C. Klein, in preparation.
4. A. Parfitt, J. L. Weller, D. C. Klein, K. K. Sakai, B. H. Marks, *Mol. Pharmacol.* **11**, 241 (1975).
5. To examine the possibility that melatonin was interfering with the measurement of LH in the radioimmunoassay, a standard curve was prepared with culture medium containing melatonin that had been incubated for 24 hours. Analysis of slope and intercept [D. Rodbard, in *Competitive Protein Binding Assays*, W. Odell and W. Daughaday, Eds. (Lippincott, Philadelphia, 1971), pp. 158–203] indicated that the resulting curve was statistically equivalent to the standard curve prepared in assay buffer alone.
6. R. W. Pelham, G. M. Vaughan, K. L. Sandock, M. K. Vaughan, *J. Clin. Endocrinol. Metab.* **37**, 341 (1973).
7. J. Arendt, L. Paunier, P. C. Sizonenko, *ibid.* **40**, 347 (1975).
8. J. E. Martin, J. Engel, D. C. Klein, in preparation.
9. I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* **87**, 1 (1970).

12 November 1975

Mixed Cultures of Sperm and Leukocytes as a Measure of Histocompatibility in Man

Abstract. *Human peripheral blood leukocyte cultures containing varying numbers of washed fresh sperm were cultured for 4 days. [^{3}H]Thymidine incorporation was used as a measure of lymphocyte transformation. Human sperm cells induce a 4- to 250-fold increase in [^{3}H]thymidine incorporation in allogeneic leukocyte cultures, but no increase was demonstrated in autologous leukocyte cultures. The response was dose-dependent with maximum stimulation obtained at 2×10^6 sperm per milliliter of culture. Seminal plasma was inhibitory in a dose-dependent fashion and as little as 0.2 microliter per 200 microliters of culture was inhibitory. The data indicate that tissues other than leukocytes can express the portion of the major histocompatibility complex responsible for allospecific lymphocyte transformation, and thus may have application in transplantation and reproductive biology.*

The mixed leukocyte culture (MLC) test is widely used as a histocompatibility test in humans and other mammalian species and is recognized as being principally dependent on differences of one genetic region in the major histocompatibility complex (*1*). In recent years, dissociated epidermal cells have also been shown to be capable of inducing lymphocyte transformation and DNA synthesis in allogeneic leukocyte cultures in the rat (*2*), the mouse (*3*), and man (*4*). Epidermal cells are the first cell type not present in peripheral blood that have been shown to be capable of inducing allospecific lymphocyte transformation. We now present data indicating that the sperm present in human ejaculates are also capable of stimulating lymphocyte transformation and DNA synthesis when added to allogeneic but not autologous leukocyte cultures (*5*). Sperm induce levels of lymphocyte transformation similar to that observed with an equivalent number of allogeneic peripheral blood leukocytes.

Fresh sperm were prepared for culture by washing two times with RPMI 1640 medium (Grand Island) that was heated to 38°C, centrifuging at 300*g* for 6 minutes and resuspending in RPMI 1640 to give a final concentration of 1×10^8 sperm per milliliter. Varying portions of the stock sperm suspension were added to leukocyte cultures to yield a final culture concentration of 0.1×10^6 to 5.0×10^6 sperm per milliliter. Leukocytes rich in mononuclear cells were prepared for culture by centrifugation of herparinized venous blood as described (*6*). The leukocyte cultures contained 20 percent autologous plasma, penicillin, and streptomycin, and were incubated in microtiter plates containing 0.2 ml of culture at 37°C in a 5 percent CO_2-humidified atmosphere (*7*). The cultures were assayed for [^{3}H]thymidine incorporation in quadruplicate on the fifth day with a multiautomated sample harvester (Microbiological Associates), and the results are expressed as counts per minute per culture ± the standard error.

Sperm from a donor (J.C., experiment 1, Table 1) was incubated with autologous leukocytes as well as allogeneic leukocytes (D.P., experiment 1). The results are expressed as the uptake of [^{3}H]thymidine in leukocyte cultures with sperm, compared to uptake of [^{3}H]thymidine of identical leukocytes cultured without sperm (leukocytes alone). In some experiments (experiments 3, 9, and 10) more than one allogeneic response was compared to a single autologous result. With fresh suspensions of sperm cells (within 2 hours of ejaculation), high degrees of lymphocyte transformation and DNA synthesis were observed when sperm were added to allogeneic leukocyte cultures, and no significant DNA synthesis was observed when sperm were added to autologous leukocyte cultures (Table 1). There was considerable variation in the degree of stimulation of allogeneic leukocytes with stimulation ratios ranging from 3.2 to 252.

The lymphocyte transformation induced by sperm was dependent on the dose, with maximal stimulation occurring between 1×10^6 and 2×10^6 sperm per milliliter of culture (Fig. 1). Seminal plasma was inhibitory to both allogeneic and autologous leukocytes stimulated with tetanus toxoid

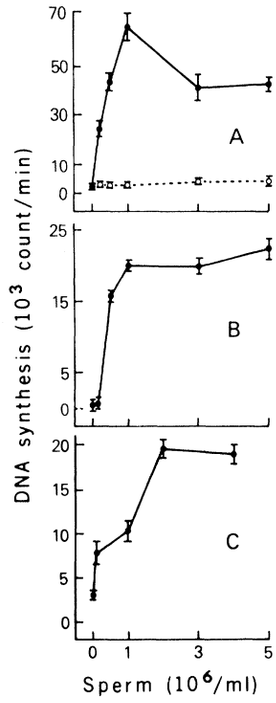
(0.4 μl per culture) (Wyeth Laboratories) (Fig. 2). As little as 0.2 μl of undiluted seminal plasma (1 percent of culture by volume) was shown to be suppressive.

Our earliest attempts to stimulate allogeneic leukocytes with frozen sperm were unsuccessful. Additional studies showed that exposing sperm suspensions to −20°C for as little as 20 minutes destroyed their stimulatory ability (data not shown).

The wide range of stimulation ratios of allogeneic leukocyte sperm cultures remains unexplained. However, the generalized ability of allogeneic leukocytes to respond to human sperm, as shown in our data, is in contrast to the highly variable and often negative responses recorded previously (*8*). These differences are probably technical, possibly related to contamination of the leukocyte cultures with seminal plasma or to differences in sensitivity of the method. Since fresh sperm are required for maximum lymphocyte transformation, the age of the sperm cells after ejaculation could conceivably be an additional factor accounting for the lack of stimulation by some sperm.

We observed low level (less than 1 percent of the response seen in leukocyte culture) incorporation of [^{3}H]thymidine in two instances when phytohemagglutinin was added to cultures containing only cells from ejaculates. This raised the question of whether ejaculates are contaminated by lymphocytes, which might account for the subsequently demonstrated transformation of allogeneic leukocytes. However, only occasional leukocytes were observed in a few of the ejaculates tested in these studies. Their numbers were too small to count, and they were predominantly polymorphonuclear cells. Since a comparable number of peripheral blood mononuclear leukocytes would be inadequate to stimulate allogeneic cells, it seems unlikely that the occasional low response with phytohemagglutinin could signify enough lymphocytes for the high degree of observed stimulation by sperm.

The fact that cells other than leukocytes are capable of stimulating allogeneic lymphocytes is not surprising, since evidence indicates that the mixed leukocyte response is driven by a product of the major histocompatibility complex (MHC). Furthermore, sperm have been shown to possess HL-A antigens (*9*). While HL-A antigens are distinct from the genetic region responsible for the mixed leukocyte response (*10*), both represent expressions of the MHC. Studies in mice reveal that another product of the MHC, the immune-response associated (Ia) product, is present on some mouse lymphocytes, macrophages, sperm cells, and epidermal cells (*11*) and that the Ia product on mouse lymphocytes may be responsible for stimulation in MLC's (*12*). The human cell types which have been shown to be capable of



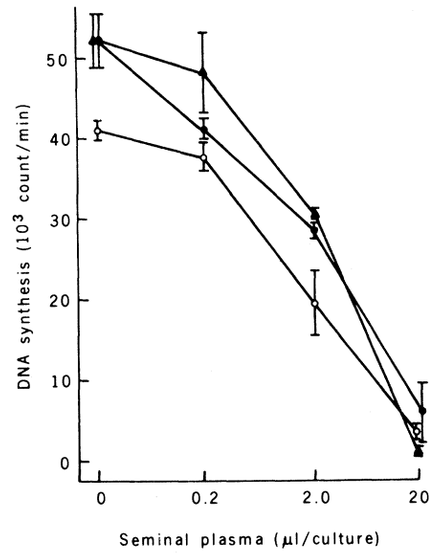


Fig. 1 (left). [^{3}H]Thymidine incorporation (mean ± S.E.) into leukocyte cultures containing varying concentrations of sperm. Allogeneic leukocytes (—) and autologous leukocytes (---) are shown for three different subjects (A, B, C). Fig. 2 (right). [^{3}H]Thymidine incorporation (mean ± S.E.) into tetanus toxoid stimulated leukocyte cultures containing varying amounts of seminal plasma. The effect of seminal plasma on two different allogeneic leukocyte cultures is shown by open and closed circles and on an autologous leukocyte culture by the closed triangles.

Table 1. [^{3}H]Thymidine incorporation into leukocyte cultures (counts per minute ± standard error).

Experiment	Autologous leukocytes				Allogeneic leukocytes			
	Sperm donor	Leukocytes alone	Leukocytes and sperm	Stimulation ratio*	Leukocyte donor	Leukocytes alone	Leukocytes and sperm	Stimulation ratio
1	J.C.	732 ± 124	1496 ± 586	2.0	D.P.	590 ± 43	2229 ± 834	3.8
2	D.P.	590 ± 43	511 ± 100	0.9	J.C.	732 ± 124	63380 ± 7507	86.6
3	B.W.	235 ± 12	206 ± 40	0.9	R.T.	243 ± 67	14503 ± 1434	59.7
	B.W.	235 ± 12	206 ± 40	0.9	B.G.	1525 ± 216	16356 ± 1576	10.7
	B.W.	235 ± 12	206 ± 40	0.9	B.P.	85 ± 28	21405 ± 875	251.8
	B.W.	235 ± 12	206 ± 40	0.9	A.A.	683 ± 241	19919 ± 583	29.2
4	I.B.	2161 ± 388	2204 ± 236	1.1	F.B.	1018 ± 86	3298 ± 1432	3.2
5	R.J.	595 ± 76	677 ± 145	1.1	F.B.	1018 ± 86	7142 ± 2233	7.0
6	B.K.	1095 ± 148	951 ± 299	0.9	J.C.	2838 ± 620	26519 ± 893	9.3
7	W.K.	613 ± 33	776 ± 137	1.3	R.C.	593 ± 87	9084 ± 723	15.3
8	R.C.	593 ± 87	1605 ± 302	2.7	W.K.	613 ± 33	6085 ± 801	9.9
9	T.M.	1428 ± 118	1943 ± 478	1.4	R.M.	651 ± 144	16117 ± 9144	24.8
	T.M.	1428 ± 118	1943 ± 478	1.4	C.J.	616 ± 162	18021 ± 2568	29.2
10	D.M.	1704 ± 377	1035 ± 95	0.6	R.M.	1954 ± 563	16998 ± 1927	8.7
	D.M.	1704 ± 377	1035 ± 95	0.6	J.A.	993 ± 85	26359 ± 4153	26.5
	D.M.	1704 ± 377	1035 ± 95	0.6	T.M.	391 ± 133	4725 ± 1616	12.1

*This stimulation ratio is the ratios of the counts of allogeneic leukocytes with sperm to the background count of allogeneic leukocytes alone.

stimulating allogeneic lymphocytes are identical to those which express the Ia product in mice—namely macrophages (*13*), lymphocytes (*13*), epidermal cells (*4*), and sperm cells.

Evidence suggests that sperm exhibit haploid expression of HL-A antigens (*9*) and possibly other products of the MHC. Such haploid expression on human sperm offers a number of theoretical and potentially practical applications in diverse fields such as transplantation and reproductive biology.

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References

1. F. H. Bach, Ed., *Immunobiology of Transplantation* (Grune & Stratton, London, 1973).
2. R. K. Main, K. D. Cochrum, M. D. Jones, S. L. Kountz, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1165 (1971).
3. R. W. Gillette, S. Cooper, E. M. Lance, *Immunology* **23**, 769 (1972).
4. W. R. Levis and A. E. Miller, *Lancet* **1972-II**, 357 (1972).
5. W. R. Levis, J. J. Whalen, R. J. Sherins, *ibid.* **1974-II**, 954 (1974).
6. W. R. Levis and J. H. Robbins, *Blood* **40**, 77 (1972).
7. J. A. Powell, J. J. Whalen, W. R. Levis, *J. Invest. Dermatol.* **64**, 357 (1975).
8. D. M. Mumford, P. B. Barsales, K. D. Ball, H. L. Gordon, *J. Urol.* **105**, 858 (1971); R. P. Erickson and D. P. Stites, *Lancet* **1975-I**, 112 (1975).
9. M. Fellous and J. Dausset, *Nature* **225**, 191 (1970).
10. E. J. Yunis and D. B. Amos, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 3031 (1971).
11. G. J. Hämmerling, G. Manup, E. Goldberg, H. O. McDevitt, *Immunogenetics*, **1**, 428 (1975).
12. C. G. Fathman, B. S. Handwerger, D. H. Sachs, *J. Exp. Med.* **140**, 853 (1974).
13. W. R. Levis and J. H. Robbins, *Transplantation* **9**, 515 (1970).

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Rapid Oral Mixing of Glucose and Saccharin by Rats

Abstract. *Within 5 minutes after initial contact rats show excessive consumption of a mixture of saccharin and glucose solutions. With a glucose solution in one bottle and the saccharin solution in another, a combination of which matches the above mixture, the rats also show large intakes. The pattern of drinking from the glucose and the saccharin bottles indicates that the rat mixes the solutions in rapid order, producing the preferred concentration of saccharin and glucose.*

It is widely recognized that many vertebrates have a "sweet tooth," and the evidence is clear that sweet-tasting substances reinforce behavior by virtue of their direct sensory effect (*1*). No example of this sweet preference is so profound as that described by Valenstein *et al.* (*2*). The normal fluid intake of rats was increased by nearly a logarithmic unit when the animals were offered a mixture of glucose and saccharin. Each liter of fluid in this standard mixture contained 1.25 g of sodium saccharin and 30 g of glucose (S + G solution). The consumption of this S + G solution in a 24-hour period occasionally exceeded the rat's body weight, which would be equivalent to an adult human male drinking more than 80 liters of fluid per day.

One concludes from the report of Valenstein *et al.* that the S + G solution is consumed in large quantities because it tastes good. They described the fluid as highly palatable, possessing a minimum of postingestional inhibiting factors. Many investigators (*3*) have subsequently used the standard S + G solution for its polydipsic effects in various experimental designs, but, as far as we know, no study has yet been reported that would clearly explain the basis of this highly unusual drinking behavior.

In an effort to delineate the basis of the synergistic action, Valenstein *et al.* conducted several experiments. Using male rats, we have replicated in this laboratory all of the experiments described in the study of Valenstein *et al.* (*2*) with essentially the same results. In support of their contention that the maximum contribution is from taste inputs and a minimum contribution is from postingestional factors, Valenstein *et al.* demonstrated that rats show no time delay in developing a high rate of consumption of S + G solution. The rats that Valenstein *et al.* used consumed an average of 10.3 ml of the solution in the first 30 minutes of contact. Our first experiment makes this point even more emphatically. Using the electronic lick circuits described below, we plotted cumulative licking for 10 minutes with 20 rats on their initial exposure to saccharin or the standard S + G solution. The characteristic neophobia or hesitation in drinking saccharin (*4*) demonstrated by rats on their first contact with the solution is eliminated when the glucose is added. The difference in cumulative licking between saccharin and the standard S + G solution is clear within the first 1 or 2 minutes of exposure. Although local lick rates did not vary between the two solutions, the steady licking (that is, the elimination of pausing) of the standard S + G solution led to increased consumption within the 10-minute session. Our conclusion was similar to that of Valenstein *et al.*; that is, the standard S + G solution is very palatable to the rat.

The results of a second experiment by Valenstein *et al.* (*2*), however, were more difficult to understand. They stated that rats drink far more of the standard S + G solution than "equivalent solutions of glucose and of saccharin presented in separate bottles." They presented 13 male rats with 0.125 percent (by weight) saccharin and 3 percent (by weight) glucose in separate bottles and observed no excessive intake; that is, the total fluid consumption never exceeded 65 ml over 24 hours. There are two possible explanations of why the rats did not consume saccharin and glucose separately as they did the standard S + G solution. (i) Rats tend to have discrete drinking bouts in which they drink either the glucose or the saccharin solution, and these were probably separated by minutes, perhaps even hours. Therefore, they would not normally mix these two solutions. (ii) If the rats did mix the 3 percent glucose and the 0.125 percent saccharin solutions, these concentrations used by Valenstein *et al.* would result in a weak S + G solution, in fact, one that would be half as concentrated as the standard S + G solution.

In our second experiment we attempted to determine if a 6 percent glucose solution and a 0.25 percent saccharin solution presented in separate bottles (a 1 : 1 mixture of these two solutions results in the standard S + G solution) would result in the polydipsia described by Valenstein *et al.* If the excessive drinking of the standard S + G solution were based on taste factors, it seems possible that in this two-bottle test the taste of the saccharin may linger long enough to interact with glucose drinking or vice versa, so that the synergy would be formed.

For our experiments 20 male Charles River albino rats were housed in individual cages and given Purina Chow freely. For 11 days the rats were given a choice between 0.125 percent saccharin and 3 percent glucose. The bottles were removed from the cages, washed, and refilled with fresh solutions each day. After this test and 4 days of unlimited water, the rats were tested for 9 days on 6 percent glucose and 0.25 percent saccharin solution simultaneously presented in two bottles. Another 4 days of unlimited water followed, and finally the rats were given a choice between the standard S + G solution and water. In all the tests the positions of the bottles were reversed daily to allow for any position preferences. The total fluid consumption for each day was determined for each rat and averaged over the 11-, 9-, and 10-day test periods. The median total intake of the 0.125 percent saccharin and 3 percent glucose solutions was 136 ml. The median intake of the 0.25 percent saccharin and 6 percent glucose was 236 ml, and the