(release of TSH) to elevated K^+ is re-established however when Ca^{++} is reintroduced in the incubation fluid (protocol No. 8026), thus showing that the absence of response in absence of Ca^{++} is not due to some irreversible cellular damage.

(3) *Effects of* L-*thyroxine Na (T4) on TSH-release induced by elevated* K^+. In the first experiment (protocol No. 7990b), a large dose of T4 (10 µg/ml) prevented the release of TSH induced by 50 meq K^+/l. In 2 subsequent experiments (protocol Nos. 8002b, 8048), pre-incubation of the pituitary tissues with 1.25 µg T4/ml prevented the release of TSH induced by 25 meq K^+/l.

Discussion. Perhaps the most striking aspect of the observations reported in this note is to be found in the similarities between the effects (TSH release) of K^+ and TRF: both require the presence of Ca^{++} for their activity in releasing TSH and thyroxine can inhibit the TSH release induced by either K^+ or TRF.

The results reported here are consistent with one of our current hypotheses that a decrease in the membrane potential of the thyrotroph cells of the adenohypophysis may be involved in the action of TRF to stimulate release of TSH.

Addendum. Since these data were submitted for publication, evidence has been obtained that elevated K^+ stimulated in vitro secretion of ACTH and LH, as measured by bioassays; also, SAMLI and GESCHWIND have reported (Prog. 49th Meet. Endocr. Soc., p. 58) that elevated K^+ stimulated secretion of LH, as measured by immunoassay[2].

Résumé. L'augmentation de la concentration en potassium (K^+) à 25 meq/l et au delà, du liquide dans lequel des hypophyses de rat sont incubées in vitro stimule la sécrétion de l'hormone thyréotrope (TSH). La présence de l'ion calcium (Ca^{++}) est nécessaire à cet effet de K^+; l'incubation dans un milieu sans Ca^{++} empêche l'effet (sécrétion de TSH) de K^+, qui est rétabli quand on réintroduit Ca^{++} dans le liquide d'incubation. La stimulation de la sécrétion de TSH due à K^+ est inhibée par la thyroxine. Les effets de K^+ sur la sécrétion de TSH sont identiques à ceux de TRF (TSH-releasing factor) hypothalamique, en ce qui concerne la présence de Ca^{++} et l'inhibition par la thyroxine.

W. VALE and R. GUILLEMIN

Department of Physiology, Baylor University College of Medicine, Houston (Texas 77025, USA), 1st June 1967.

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A Qualitative Difference between Certain H-2 and Non-H-2 Antigens Responsible for a Similar Graft-Rejection Rate

The establishment of congenic (isogenic resistant) lines of mice[1] made it possible to compare the relative strength of antigens controlled by individual histocompatible (H) loci. By expressing the antigenic strength in terms of graft rejection rate, one strong and several weak H-loci were defined. The strong locus designated H-2 is a complex pseudoallelic series whose individual pseudoalleles control several antigenic specificities[2]. In the usual H-2 incompatible donor-host combinations the time taken for the rejection of first-set skin grafts does not exceed 14 days. When, however, the spectrum of H-2 antigens representing the target of allograft reaction is limited, the graft rejection time may be prolonged[3]. In contrast to this, a cumulative effect of weak (non-H2) loci was described[4].

MCKHANN[5] compared strong and weak histocompatibility barriers by means of graft sensitivity induced by spleen cells. In the H-2 system, sensitization appeared rapidly and persisted for a shorter time than in H-3 where it appeared more slowly. The ease of tolerance-induction usually appears inversely proportional to the graft rejection rate. In adult mice, induction of tolerance across H-2 barrier requires a prolonged administration of large doses of spleen cells[6], whereas a single dose is sufficient when H-3 barrier is to be overridden[7].

We were interested in whether the H-2 and non-H-2 antigens are qualitatively similar when chosen so that they represent a comparable histocompatibility barrier.

In a combination differing at 2 'weak' loci, i.e. donor B10.LP ($H\text{-}1^c$, $H\text{-}2^b$, $H\text{-}3^b$) and recipient B10.BY ($H\text{-}1^d$, $H\text{-}2^b$, $H\text{-}3^a$) the average skin graft rejection time is about 17 days, whereas in a particular H-2 incompatible combination (i.e. donor B10.A[RII]) ($H\text{-}2^h$) and recipient (B10.D2 × B10.BR)F1 ($H\text{-}2^d/H\text{-}2^k$) where according to the data given by SNELL et al.[2], the antigenic target is presumably only antigen 2 (D^b), it amounts to 20 days.

The skin grafting technique was in principle that described by BILLINGHAM and MEDAWAR[8]; the first macroscopic examination followed on day 7 postgrafting and then daily. The day of appearence of an essential cosmetic defect (which does not tend to improve on following days) is given as the day of graft rejection. The criterion of a 'permanent tolerance' is the survival of second skin graft on recipient tolerating the first graft for more than 100 days.

Recipients of skin grafts were pretreated with a single dose of 5×10^7 or 8×10^8 of viable spleen cells. Test grafting followed on day 4, 10 or 40 after the lower dose and on day 3 after the higher one. In the case of H-2 incompatibility, the dose of 5×10^7 cells led to an accelerated rejection of 2 out of 5 grafts made on the fourth day and of all grafts made 10 and 40 days after the injection. In the (H-1 + H-3) system, a similar dose of spleen cells

[1] G. D. SNELL, J. Genet. *49*, 87 (1948).
[2] G. D. SNELL, G. HOECKER, D. B. AMOS and J. H. STIMPFLING, Transplantation *2*, 777 (1964).
[3] J. KLEIN, Folia biol., Praha *12*, 168 (1966).
[4] C. F. MCKHANN, Nature *201*, 937 (1964); J. KLEIN, Folia biol., Praha *11*, 169 (1965).
[5] C. F. MCKHANN, Transplantation *2*, 613 (1964).
[6] F. SHAPIRO, C. MARTINEZ, J. M. SMITH and R. A. GOOD, Proc. Soc. exp. Biol. Med. *106*, 472 (1961).
[7] C. F. MCKHANN, Transplantation *2*, 620 (1964).
[8] R. E. BILLINGHAM and P. B. MEDAWAR, J. exp. Biol. *28*, 385 (1951).

Response of mice to a test skin graft given at different intervals after pretreatment with viable spleen cells

Interval (days)	Fate of test skin grafts (No./total) after stated cell dose									
	(H-1 + H-3)					H-2 (antigen 2 only)				
	5×10^7 [a]		8×10^8 [b]			5×10^7 [a]		8×10^8 [b]		
	FSR	SSR	Tolerance [c]	FSR	SSR	FSR	SSR	Tolerance [c]	FSR	SSR
3	–	–	8 [d]/15	7/15 (15–20.3–27) [e]	0/15	–	–	0/15	0/15	15/15 (8–8.8–10) [e]
4	5/5 (12–14.4–16) [e]	0/5	–	–	–	2/5 (15–15.5–16) [e]	3/5 (11–11.3–12) [e]	–	–	–
10	6/6 (12–13.8–15) [e]	0/6	–	–	–	0/5	5/5 (8–9.0–10) [e]	–	–	–
40	0/6	6/6 (9–9.2–10) [e]	–	–	–	0/6	6/6 (10–10.8–12) [e]	–	–	–

FSR, first set rejection; SSR, second set rejection; [a], 5×10^7 i.p.; [b], 1.10^8 i.v. + 7.10^8 i.p.; [c], graft rejection time at least 30 days; [d], permanent survival in 3 of these; [e], minimum, average and maximum rejection time.

did not affect the skin grafts made after 4 or 10 days and accelerated rejection of only those made after 40 days.

The dose of 8×10^8 spleen cells induced second-set rejection of all H-2 incompatible grafts (transplanted after 3 days) and of none of the (H-1 + H-3) incompatible grafts where 3 were even permanently tolerated (Table).

From these results it may be concluded that in spite of a comparable first set graft rejection time they induce, the H-2 and non-H-2 antigens may turn out to be qualitatively different when tested by means of skin grafting after antigenic pretreatment with viable spleen cells. Similar cell doses can induce sensitization when a single H-2 antigen is involved, whereas prolonged survival or tolerance may result in a weak non-H-2 system.

Zusammenfassung. Gezeigt wird, dass «schwache» Histokompatibilitätsantigene weniger sensibilisieren als «starke», auch wenn beide eine quantitativ ähnliche Transplantationsbarriere darstellen. Auf qualitative Unterschiede zwischen diesen Antigenen nach Vorbehandlung des Rezipienten mit lebenden Milzzellen von Mäusen wird hingewiesen.

I. HILGERT

Czechoslovak Academy of Sciences, Institute of Experimental Biology and Genetics, Praha 4 (Czechoslovakia), 28th October 1966.

On the Antimutagenic Effect of Spermine

It has been reported [1] that the inclusion of the aliphatic tetramine, spermine, at a non-inhibitory concentration (150 μg/ml) in the medium significantly decreases spontaneous mutation to streptomycin resistance in *Escherichia coli* and *Staphylococcus aureus*, and decreases spontaneous reversion of a tryptophane-requiring strain of *E. coli*. The antimutagenic effect to streptomycin resistance in *E. coli* is claimed to be even more apparent on induced mutation when spermine is present either during treatment with caffeine [1], or during growth prior to UV-irradiation [1]. Subsequently, the same workers [2] have demonstrated a similar antimutagenic effect on mutation to streptomycin resistance when spermine is present during growth of Yanofsky's mutator gene-containing strain of *E. coli*, and during treatment of a wild type strain of *E. coli B* with 2-aminopurine.

Observations that spermine reacts with DNA [3], and protects DNA against breakage by hydrodynamic shear [4], suggest that the binding of spermine to DNA could be involved in the antimutagenic effect. The present paper reports preliminary experiments designed to detect an antimutagenic effect of spermine on spontaneous reversion of nucleic acid base analogue-induced and acridine induced *r*II mutants of *E. coliphage T4*: no antimutagenic effect of spermine is apparent.

The mutants used were *N*11, which is 5-bromouracil induced [5], occurs at a 5-bromouracil 'hot spot', and is revertible by 5-bromodeoxyuridine but not by proflavine or 5-aminoacridine [6]; and *FC*47, which is proflavine-induced, and revertible by proflavine, but not by base-analogue type mutagens [7].

[1] H. G. JOHNSON and M. K. BACH, Nature *208*, 408 (1965).
[2] H. G. JOHNSON and M. K. BACH, Proc. natn. Acad. Sci. USA *55*, 1453 (1966).
[3] M. MANDEL, J. molec. Biol. *5*, 435 (1962).
[4] D. KAISER, H. TABOR and C. W. TABOR, J. molec. Biol. *6*, 141 (1963).
[5] S. BENZER and E. FREESE, Proc. natn. Acad. Sci. USA *44*, 112 (1958).
[6] A. ORGEL and S. BRENNER, J. molec. Biol. *3*, 762 (1961).
[7] F. H. C. CRICK, L. BARNETT, S. BRENNER and R. J. WATTS-TOBIN, Nature *192*, 1227 (1961).