of transit from G_0 to S depends on nuclear size—relate to current models? There are two major kinds of models, deterministic and probabilistic. Kinetics do not always permit a choice between them (*16*). The present result is deterministic; an individual cell's physical characteristics affect its cycle kinetics.

According to the transition probability model (*7*), G_0 cells are not distinct from cycling cells; they simply have a longer average A phase, defined as a "waiting" phase in G_1, from which cells move to S with a constant probability per unit time (under a given set of conditions). This model has been applied to kinetics of escape from the serum-derived quiescent state (*17*). Hence the present experiments are relevant to assessing this model's validity.

The transition probability model asserts that it is not possible to determine a priori which G_1 cells will first enter S phase. Our data are contrary to this model. Cells were observed to be unequal in their capability to traverse the cell cycle. The data must invalidate at least one of the specified properties of the transition probability model; either the transition probability or the duration of the B phase (the proposed invariant portion of the cell cycle) depends on nuclear size and is not the same for all cells, as the model postulates. Moreover, if nuclear size affects the duration of the B phase, then a very significant amount of variability in cell-cycle durations is incurred in B as well as in A.

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Linkage of Loci Controlling Alloantigens on Red Blood Cells and Lymphocytes in the Horse

Abstract. *A system of equine lymphocyte alloantigens designated ELA, is identified, and it is shown that the locus or loci controlling these markers must be closely linked to the locus controlling markers in the A system of horse blood groups. Among 29 offspring in two stallion families there was evidence for one recombinant. Lod scores for linkage between the A and ELA loci in the two families were 3.61 and 3.33, respectively, for θ equal to 0.*

Although previous reports (*1*) have indicated that alloantigenic markers on horse lymphocytes are inherited as dominant traits and are not expressed on red blood cells, little information concerning their potential allelic relationships has been published. We now have evidence that four such markers, designated E1, E2, E3, and E4, are probably coded for by a single series of allelic genes, and that the locus bearing these alleles is closely linked to the A locus of horse blood groups. The A locus is known to bear at least eight alleles (*2*). The evidence for the segregation of E1, E2, E3, and E4 as the products of allelic genes and of their linkage to markers in the A system of horse blood groups is based on two sire families. One family consisted of a Shetland Pony stallion, 10 mares, and their 17 offspring. The other consisted of an Appaloosa stallion, 12 mares, and their 12 offspring.

Blood samples collected in 10 ml acid-

Table 1. Inheritance of erythrocyte antigens A_1 and Z and lymphocyte antigens E1, E2, E3, and E4 in a Shetland Pony family. The presence of the antigen is signified by +; the absence by −. An allele which may also have been contributed by the dam is shown in parentheses. Abbreviation: ND, not determined.

Horse identification	Sex	A_1	Z	E1	E2	E3	E4	Probable sire contribution
Sire		+	+	+	+	−	−	
Dam Py13		−	+	−	−	−	ND	
Offspring Py64	♀	+	−	+	−	−	−	A_1, E1
Offspring Py73	♀	−	+	−	+	−	−	(Z), E2
Dam Py16		−	−	−	−	ND	ND	
Offspring Py56	♂	+	−	+	−	+	−	A_1, E1
Offspring Py63	♂	+	−	+	−	+	−	A_1, E1
Dam Py19		−	−	−	−	ND	ND	
Offspring Py65	♀	−	+	−	+	−	−	Z, E2
Dam Py26		+	−	−	−	−	−	
Offspring Py59	♀	+	+	−	+	−	−	Z, E2
Offspring Py76	♂	+	+	−	+	−	−	Z, E2
Dam Py33		−	−	−	ND	ND	ND	
Offspring Py60	♂	+	−	+	−	+	−	A_1, E1
Dam Py35		−	−	−	−	ND	ND	
Offspring Py70	♀	+	−	+	−	−	−	A_1, E1
Offspring Py75	♀	+	−	+	−	−	−	A_1, E1
Dam Py38		+	−	+	−	−	−	
Offspring Py68	♂	−	+	−	+	−	−	Z, E2
Dam Py42		−	−	+	−	ND	ND	
Offspring Py61	♀	+	−	+	−	−	−	A_1, (E1)
Offspring Py67	♀	−	+	+	+	−	−	Z, E2
Offspring Py74	♂	+	−	+	−	−	−	A_1, (E1)
Dam Py44		−	−	−	−	−	−	
Offspring Py62	♂	+	−	+	−	−	−	A_1, E1
Offspring Py69	♀	+	−	+	−	−	−	A_1, E1
Dam Py49		−	−	−	−	+	−	
Offspring Py78	♀	+	−	+	−	−	−	A_1, E1

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Table 2. Inheritance of erythrocyte antigens A_1 and A′ and lymphocyte antigens E1, E2, E3, and E4 in an Appaloosa family. The presence of the antigen is signified by +; the absence by −. An allele which may also have been contributed by the dam is shown in parentheses. Abbreviation: ND, not determined.

Horse identification	Sex	A_1	A′	E1	E2	E3	E4	Probable sire contribution
Sire 49		+	+	−	−	+	+	
Dam 52		+	−	−	−	−	−	
Offspring 53	♂	−	+	−	−	−	+	A′, E4
Dam 54		−	+	−	−	−	−	
Offspring 55	♂	+	−	−	−	−	+	A_1, E4
Dam 56		+	−	−	+	−	−	
Offspring 57	♂	+	+	−	+	−	+	A′, E4
Dam 58		+	−	−	−	−	−	
Offspring 59	♂	−	+	−	−	−	+	A′, E4
Dam 60		+	−	−	−	−	+	
Offspring 61	♀	+	−	−	−	+	−	(A_1), E3
Dam 66		+	+	−	+	+	−	
Offspring 67	♀	−	+	−	ND	−	+	(A′), E4
Dam 71		+	−	−	−	−	−	
Offspring 72	♂	+	−	−	−	+	−	(A_1), E3
Dam 73		+	−	−	−	−	−	
Offspring 74	ND	+	−	−	−	+	−	(A_1), E3
Dam 75		+	−	−	−	−	−	
Offspring 76	♂	+	−	−	−	+	−	(A_1), E3
Dam 77		+	−	−	−	−	−	
Offspring 78	♀	−	+	−	−	−	+	A′, E4
Dam 79		+	−	−	−	−	+	
Offspring 80	♀	+	+	−	−	−	+	A′, (E4)
Dam 81		+	−	−	−	+	+	
Offspring 82	♀	+	−	−	−	+	−	(A_1), (E3)

citrate-dextrose Vacutainer tubes served as a source of lymphocytes for the lymphocytoxicity tests and red cells for the blood typing tests. Lymphocyte typing reagents were obtained from multiparous mares, as well as from antiserums produced by immunizing horses with whole blood or with isolated lymphocytes and platelets.

Lymphocytes were harvested and typed in accordance with the methods described by Terasaki and Park (*3*). Red cells were typed in accordance with procedures described by Stormont *et al.* (*4*), utilizing reagents prepared in this laboratory. Although all the horses in this study were typed with our complete battery of equine blood-typing reagents and with reagents which define at least 14 alloantigenic markers on horse lymphocytes, the present report is concerned only with the red cell antigenic markers known as A_1, A′, and Z, and with the lymphocyte markers designated here as E1, E2, E3, and E4. The alloantigenic markers on horse red cells do not react with or absorb the antibodies specific for the alloantigenic markers on horse lymphocytes, or the converse.

The Shetland Pony stallion (Table 1) possessed erythrocyte markers A_1 and Z and lymphocyte markers El and E2. As shown in Table 1, each of these pairs of markers segregated as alternatives in the 17 offspring. However, without exception, each time an offspring inherited A_1 from the stallion it also inherited E1, and each time an offspring inherited Z from the stallion it also inherited E2. Thus, 11 of the 17 offspring inherited A_1 and E1 together, whereas the remaining six inherited Z and E2 together, thereby providing evidence for linkage between the two pairs of markers. The lod score (*5*) for linkage of the A locus markers with the locus for E1 and E2 was 3.61, with $\theta = 0$.

The Appaloosa stallion (Table 2) possessed erythrocyte markers A_1 and A′, and lymphocyte markers E3 and E4. As may be seen in Table 2, five offspring inherited the stallion's A_1 and E3 together, whereas another six offspring inherited his A′ and E4. There was one exceptional offspring, number 55, which inherited A_1 and E4 together from this stallion. The exception is interpreted as a recombinant involving crossing-over between the A locus and the locus controlling E3 and E4. The lod score (*5*) for linkage of these loci is 3.33, with $\theta = 0$.

While there is nothing in the segregation and linkage data of this report that would conclusively prove that the contrasting pair, E1 and E2, of the Shetland Pony stallion (Table 1) are coded for by the same series of alleles involved in the control of E3 and E4 of the Appaloosa stallion (Table 2), population data suggest that this is the case. For example, we have yet to encounter in typing 204 horses for these four markers a horse possessing more than two. Thus, the simplest explanation is that the four markers are coded for by a series of codominant alleles of the equine alloantigenic marker system (ELA), ELA^{E1}, ELA^{E2}, ELA^{E3}, and ELA^{E4}.

While we have no direct proof that the ELA locus falls within the major histocompatibility complex (MHC) of horses, it is significant that in humans, mice, pigs, and chickens, there is evidence that at least one locus controlling alloantigenic markers on erythrocytes is within the MHC of those species (*6*). In any event, the linkage data presented here should be of major interest to others engaged in studies of alloantigenic markers on horse lymphocytes because many of those workers are also engaged in studies of horse blood groups.

It is of additional interest that the Shetland Pony stallion of Table 1 is known to be heterozygous for a pair of alleles at each of four blood group loci (K, P, Q, and T), in addition to A, and a pair of alleles at each of seven loci controlling electrophoretic markers, namely, serum albumin, esterase, prealbumin, transferrin, red cell carbonic anhydrase, catalase, and phosphoglucomutase. He is also heterozygous for coat color and the dominant gene controlling the tobiano spotting pattern, the latter locus being closely linked to that controlling albumin phenotypes (*7*). With the exception of the locus controlling the A system markers A_1 and Z, there has been no indication of linkage between the locus controlling E1 and E2 of this stallion and any of the other loci for which he is known to be heterozygous.

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Retrograde Amnesia for Old (Reactivated) Memory: Some Anomalous Characteristics

Abstract. *Old memory, when reactivated by cue exposure, was disrupted by mild or deep hypothermia treatments. New memory was impaired only by deep cooling. Moreover, old but not new learning showed spontaneous recovery. Old reactivated memory may be qualitatively different from newly acquired memory.*

That old memories are more stable and less susceptible to disruption than recently acquired learning has been a widely accepted proposition since the late 19th century, when Ribot, on the basis of human amnesia findings, formulated his "law of regression" (*1*). Although agreement on Ribot's principle is not universal (*2*), many reports of experimentally induced amnesia in animals have been consonant with the view that memory resists disruption by traumatic events as a function of time (*3*). Several recent studies, however, suggest that the degree of activation of memory may determine its vulnerability to insult more than the age of the information does (*4, 5*). Other evidence suggests not only that new and old (reactivated) memories can produce comparable interference effects (*6*), but also that both classes of memories may be enhanced by strychnine sulfate (*7*). We report here data substantiating several similarities as well as dissimilarities of new and old target memories subjected to amnesic treatment.

Because the induction of amnesia for cue-reactivated older memories has not always been obtained (*8*), we initiated our study in an attempt to replicate and extend the original demonstration (*4, 9*). Hypothermia was chosen as the amnesic agent since, like electroconvulsive shock, it is highly effective in producing retrograde amnesia for new learning (*10, 11*). In our first experiment, 36 adult male Holtzman rats (295 to 380 g) received one-trial training in a black-white passive-avoidance chamber (45.5 by 17.5 by 23.5 cm). When the rats crossed into the black compartment, they received an inescapable 1-second 150-V scrambled footshock.

After the training session, the animals were randomly divided into four groups of nine each; the experimental treatment was given 24 hours later. For three groups, a brief 30-second exposure to the black "fear" compartment was presented to reactivate the memory trace of passive-avoidance training. Immediately after cue exposure, they were subjected to mild or severe hypothermia treatment or no treatment and were then returned to their home cage. Hypothermia was induced by immersing restrained rats to neck level in water at 4°C until body temperatures were reduced to approximately 30.0° or 21.0°C for the mild and severe conditions, respectively. Mild body cooling was intended as a control for the general effects of stress per se (*10, 12*). The third group, which did not receive cold treatment, was used to assess the reactivation or extinction effect of the brief cue exposure. A fourth group of animals, included to establish a retention baseline, did not receive cue exposure or hypothermia treatment, but was simply transported to the experimental room and handled for 30 seconds. Twenty-four hours after experimental treatment all subjects received a passive-avoidance test to assess retention of the original training experience. Latency to enter the compartment previously associated with shock provided the index of memory.

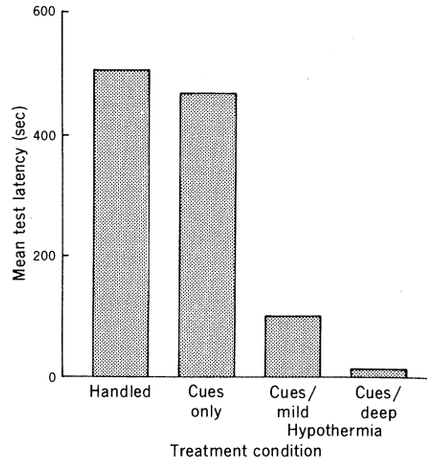

Fig. 1. Mean cross-through latency on a passive avoidance test trial 24 hours after experimental treatments. Considerable loss of retention is indicated by a rapid cross-through response.

Prior to punishment, the groups did not differ in their response latencies ($F < 1.0$). A one-way analysis of variance on the retention-test latencies (*13*) confirmed an effect of the experimental treatments ($P \leq .002$) (Fig. 1). As expected, the brief cue exposure alone had little, if any, effect on the retention normally observed 48 hours after passive-avoidance training. In contrast, the severe-hypothermia group exposed to the cue showed substantial memory loss, as reflected by test scores significantly lower than those of either the cues-only or handled controls ($P \leq .01$). To our surprise, the old reactivated memory was also disrupted by brief cooling ($P \leq .05$) with the resultant decrement indistinguishable from that produced by the more severe treatment. The severity of memory loss is reflected in the finding that the test latencies of both of these groups approached their respective training latencies.

The findings of a memory deficit following the combined cue exposure and hypothermia treatment substantiates previous reports (*4, 5*) that retrograde amnesia may be produced for old memories brought back to an active state. Other research indicates that brief cooling, even when administered immediately after acquisition, is not sufficient to produce retrograde amnesia (*10, 12*). Accordingly, as a check on the possibility that there may be some characteristic difference in the susceptibility to disruption of old and new memories, we conducted a direct comparison of the effect of deep and mild hypothermia on both classes of memories.

Fifty-six adult male rats received one-trial passive-avoidance training as in experiment 1. In the new learning condition, two groups of animals ($N = 11$ each) received either deep or mild hypothermia treatment within 30 seconds of the end of footshock. The disruptibility of old memories was assessed with an additional two groups of animals ($N = 11$ each) which were cooled for either a prolonged or a brief period, but only after a 24-hour delay. Both of these latter groups received a brief 30-second exposure to the fear cues of the black chamber prior to cold water immersion to produce reactivation of the target memory. As a control for systemic effects of the cold water treatment, two

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