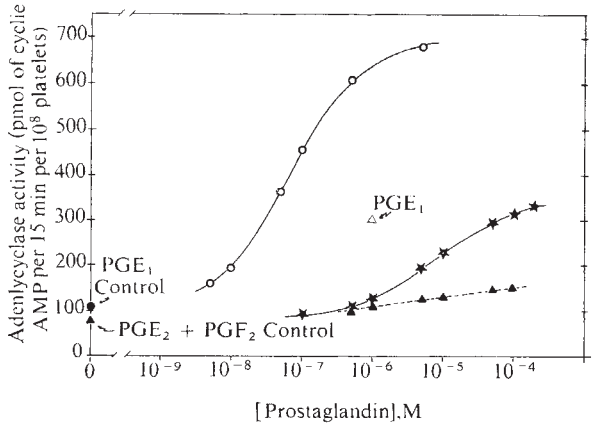

Fig. 4 The effects of PGE_1 (○), PGE_2 (★), and $PGF_{2\alpha}$ (▲) on washed human platelet adenyl cyclase. The PGE_1 data were obtained using different platelets from the PGE_2 and $PGF_{2\alpha}$ data. For reference, a single concentration of PGE_1 (△) was assayed in the PGE_2–$PGF_{2\alpha}$ platelets. In other experiments not reported, PGE_2 and $PGF_{2\alpha}$ were studied at concentrations as low as 10^{-8} M with results similar to PGE, (M.D). At PGE_1 concentrations above 10^{-5} M, a decreased output of cyclic AMP has been observed (unpublished data). It has not been possible to show this effect with PGE_2, possibly because of difficulties in getting high enough concentrations in solution.

Recent studies have suggested the existence of a regulatory or feedback mechanism of platelet function in which prostaglandins, produced and secreted in response to thrombin, may stimulate platelet adenyl cyclase to produce cyclic AMP and thereby modulate the release and aggregation processes (refs 6, 15 and M.D., unpublished data). Our findings that both PGE_2 and PGE_1 stimulate adenyl cyclase supports this hypothesis, especially as we found that presumably physiological levels of PGE_2 (refs 5, 6) were stimulatory.

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Delayed fertilisation in senescent golden hamsters

REPRODUCTIVE senescence in the female golden hamster occurs after 14 months of age and is characterised by a significant decline in the production of offspring[1]. Recent studies in our laboratory concerned with the cause(s) of this decline have revealed large numbers of non-viable ova in aged hamsters before implantation[2] and a developmental delay of approximately 12 h in preimplantation and implantation stages of embryos from senescent hamsters when compared with young females at the same hour of pregnancy[3,4]. Since the elapsed time for the union of gametes is important for normal fertilisation and development we examined ova for the onset of fertilisation in young and aged hamsters.

One hundred and twenty young virgin (3–5 months old) and 170 multiparous senescent (14–17 months old) hamsters, cycling regularly, were mated with young males (3–6 months old) at the onset of behavioural oestrus. Ovulation was considered to occur 8 h from the onset of oestrus[5] and all developmental stages are based upon this assumption. All animals were from the Oregon colony, except for 72 senescent females from commercial breeders. Ova collected from 1 h through 12 h and at 1, 1½, 2, 2½, 3 and 3½ d intervals, were either mounted *in toto* and examined with differential interference contrast microscopy (Nomarski optics) or compressed, fixed and stained with lacmoid.

Most of the ova from senescent females showed a 2 to 5 h delay in fertilisation compared with young females (Table 1). The delay was not merely an inbred characteristic of females from the Oregon colony, since aged females from two commercial animal farms demonstrated the same trait.

Although there seemed to be a short delay in the completion of ovulation in the senescent hamster 1 h after ovulation (10.5 ± 1.12 compared with 13.8 ± 0.91 for young females), there was no statistical difference in the number of ova at 2 h (12.8 ± 2.25) compared with ova from young hamsters 1 h after ovulation. The total number of ova recovered from young and senescent females was similar throughout the first 12 h (young 13.2 ± 0.33, senescent 12.1 ± 0.38), but a real difference was apparent when the number of normal-appearing ova was determined (young 12.6 ± 0.40, senescent 10.9 ± 0.30); large numbers of non-viable ova were found in the aged hamsters. Some ova were apparently defective at the onset of ovulation since they were in an advanced state of deterioration at the time fertilisation normally occurs in young females. Other fertile ova of normal appearance stained with lacmoid revealed anomalies commonly associated with a delay in fertilisation, such as fragmented pronuclei (6 ova), dispermy (3 ova), trispermy (1 ovum) and androgenesis (1 ovum). On examining zygotes

Table 1 Normal ova and their stage of fertilisation from young and senescent hamsters

Hours after ovulation	Ova penetrated by spermatozoa (Percentage)		Ova with second polar body (Percentage)		Ova with second* polar body and male and female pronuclei (Percentage)	
	Y	S	Y	S	Y	S
2	—	0.0	—	0.0	—	0.0
3	44.2	16.7	29.5	8.3	0.0	0.0
4	90.1	37.3	77.5	25.3	0.0	0.0
5	100	40.9	94.3	29.6	75.0	1.6
6	—	51.5	—	36.6	—	20.9
7	—	78.0	—	65.9	—	41.5
8	100	94.4	100	78.9	100	73.7
9	—	80.3	—	78.9	—	78.9
10	100	72.3	100	69.1	100	69.1
11	84.5	94.1	84.5	91.2	84.5	91.2
12	96.1	100	96.1	98.4	95.4	91.1

Y, Young.
S, Senescent.
* Ova stained with lacmoid.

from the uterus, we found that approximately 40% of the ova from senescent females were incapable of implantation so that preimplantation loss seems a major factor. Unfertilised ova made up a small percentage of the non-viable ova (1.4% compared with 1.3% for young females); therefore, it is conceivable that the delay in fertilisation was sufficient to have caused some embryonic loss.

Researchers have shown that delaying fertilisation in young golden hamsters by artificially inseminating[6] or naturally mating females[7] 3 h after ovulation, results in considerable embryonic loss (14% to 61%). In both studies capacitation of the spermatozoa would have required between 2 and 4 h (ref. 8); therefore, the ova were between 5 and 7 h old before fertilisation. In our study many ova from aged hamsters were not fertilised until 5–8 h after ovulation.

Spermatozoa may be slowed in their ascent through the aged reproductive tract, although oviducts compared from several young and senescent females allowed to breed for 1 h from the beginning of coitus revealed no difference in the approximate number or location of spermatozoa. No spermatozoa were detectable, however, in ampullae of oviducts of either group. A more probable cause for the delay is prolonged capacitation. This may explain why some ova from senescent hamsters in the present study are fertilised at a time when hamster spermatozoa have been shown to be no longer viable[9]. Raised levels of plasma progesterone have recently been reported in some senescent hamsters during oestrus and the first day of pregnancy[10]. This may indicate that the ageing hamster uterus is less capable of binding progesterone, a condition noted *in vitro* with the senescent rabbit uterus[11]. A refractory uterus could be causing a delay in fertilisation in hamsters since subcontraceptive levels of progesterone may delay fertilisation in rabbits[12] either by slowing spermatozoa migration through the reproductive tract and/or by prolonging capacitation[13,14].

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Abnormal potassium conductance associated with genetic muscular dystrophy

DYSTROPHIC diseases not only lead to progressive deterioration of muscle, but alter several properties of the erythrocyte surface[1-4], and permeability of various other membranes[5-7]. Muscular dystrophies are also associated with defects in the nervous system, so that a neurogenic aetiology[8] has been suggested, there being reports of peripheral neural dysfunction[9], increased frequency of mental retardation[10] and defective auditory acuity[11].

Many of these non-muscular (systemic) features of the dystrophic condition are associated with cellular membranes; indeed dystrophic muscle itself has an abnormal membrane composition[12] and greater than normal calcium-linked ATPase activity in the sarcoplasmic reticulum[13]. Moreover, dystrophic muscle exhibits a significantly altered ionic content and abnormally large potassium conductance[14], although these attributes may only reflect the characteristic degeneration of such tissue. I report here that diverse membrane systems in dystrophic animals and humans conduct potassium more rapidly than in the case of normal subjects. This higher conductance could, in principle, account for the disease process.

Table 1 shows passive K^+ efflux from erythrocytes from normal subjects and patients with Duchenne muscular dystrophy where the rate of efflux is about five times greater. In other experiments, the cardiac glycoside, ouabain, was without influence on the efflux and addition of the K^+-conducting antibiotic, valinomycin, stimulated it. The extent of K^+ loss in the presence of valinomycin, suggests that there is no consistent difference between the K^+ content in normal and dystrophic erythrocytes.

Table 1 Efflux of potassium from human erythrocytes

Source of cells	No. of subjects	Potassium efflux (nmol per min per 10^8 cells)
Normal subjects	8	21.6 ± 2.4
Duchenne dystrophy patients	7	107.9 ± 7.5

Results are expressed as mean and standard error of the mean. Samples of venous blood were drawn into a heparinised tube, cells were sedimented at 1,500*g*, resuspended in isotonic saline, washed twice and finally resuspended in about one-tenth of the original volume of saline. All operations were carried out at 0° C. Potassium efflux was measured at 20° C with a Corning model 476132 potassium electrode which exhibited an 80-fold selectivity of potassium as compared with sodium. The medium contained 360 mM sucrose, 15 mM NaCl and 0.1 mM KCl in a volume of 10 ml to which was added 0.4 ml of an erythrocyte suspension containing approximately 10^9 cells per ml.

Figure 1 shows light scattering changes after addition of potassium acetate to suspensions of mouse liver (*a*) and brain (*b*) mitochondria. Normal mitochondria from both organs are relatively impermeable to K^+ whereas those from dystrophic animals took up a significant amount. I have observed a similar increase in K^+ conductance with liver mitochondria from a second strain of dystrophic mice (129ReJ-dy) and from nutritionally dystrophic rats maintained on a vitamin E-deficient diet. This last observation suggests that membrane dysfunction may be the basis for similarity between genetic and nutritional dystrophy. Addition of Na^+ and Li^+ acetate does not result in significant changes in light scattering by mitochondria from either normal or dystrophic animals. In dystrophic mitochondria, the uptake of K^+ is probably energy-linked, as subsequent addition of 2 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, an uncoupler of oxidative phosphorylation, returns the absorbance to its initial value.

Thus there is greater than normal conductance of K^+ across erythrocyte, liver mitochondrial and brain mitochondrial membranes in the dystrophic state. Since none of these tissues seem to degenerate as muscle does, the genetic defect in muscular dystrophy may give rise to membranes, throughout the organism, which are leaky to K^+, and muscle deterioration may be a consequence of that leak. This may occur through defective excitation-coupling where rapid K^+ movement might serve to collapse charge dislocation required for a cycle of membrane depolarisation–repolarisation. In this connection, it is interesting that the T tubular system, a major locus for K^+ transport to the muscle interior, has been shown to be structurally altered in muscular dystrophy[15].