

interesting evolutionary implications, some of which have been discussed in the context of lower organisms (20).

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- The descriptions of the fibroblast cultures are as follows:

Designation	Age (years)	Passages used	HGPRT (nmole/hr-mg)
<i>Normal donors</i>			
GM 302	0.8	7 to 12	103
GM 316	7	4 to 10	88
GM 407	10	9 to 15	100
GM 409	12	9 to 14	107
<i>Lesch-Nyhan donors</i>			
GM 29	0.7	9 to 14	< 2
GM 68	2	15 to 18	< 2
GM 152	9	11 to 16	< 2
GM 158	3	10 to 15	< 2
GM 159	10	12 to 17	< 2
GM 177	6	10 to 16	< 2
GM 537	12	9 to 15	< 2
S.S.	3	3 to 10	< 2

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The calves were then followed by ship as they left the lagoons. These methods require tracking by ship and aircraft, which is not only expensive, but also practical only in areas with good port and air facilities and where good weather is common. Such requirements are not met in the Bering Sea.

Depth recorders (3) and mechanical depth-time recorders (4) have been placed on the antarctic Weddell seal, *Leptonychotes weddelli*. When the seals, who were diving under ice, returned to the known ice holes in order to breathe, the instruments could be removed and the data recovered. The depth recorders provided only the maximum depth of all the dives made while the recorder was attached and therefore did not provide enough information for our purposes. The depth-time instrument's 4-hour recording duration was too short for the northern fur seal study.

We now present some preliminary results gathered with a new instrument. The instruments we designed and built for this project could continuously record every dive for 8 days (5). A depth transducer plots a record on pressure-sensitive paper, which is transferred from one spool to another by an electric motor. The mechanism is housed in an aluminum waterproof container weighing 650 g and measuring 5 cm in diameter by 17 cm long. The instruments were attached to a harness worn by the seals. The two measures (depth and time) provide a variety of information, such as the time feeding begins after the seals depart from the rookery, circadian activities, diving effort, and diving depth.

Lactating females seemed the best animals to equip with this recorder since (i) they form the largest segment of the population, (ii) at the time of our study (summer) they spend more time at sea than on shore, and (iii) their movements ashore are predictable in space and time (6). This third point was important for ensuring a low probability of instrument loss. In essence, the females transported our instruments and gathered data for us while we waited ashore for their return.

Our data are summarized as the number and depth of dives observed (Table

Northern Fur Seal Diving Behavior: A New Approach to Its Study

Abstract. A new type of depth-time recorder was used to monitor behavior of fur seals at sea. During 608 hours, 2957 dives were recorded for four animals. The deepest dive was 190 meters, and the longest submersion was 5.6 minutes.

Recently, it became necessary to assess the behavior of the northern fur seal, *Callorhinus ursinus*, in the Bering Sea in order to predict the effects of potential oil spills near their breeding grounds. Remote acquisition of information about diving behavior has been done by only a few investigators. Evans (1) surveyed

some small whales by attaching radio transmitters to their dorsal fins. The signal was monitored with a receiver on a nearby ship or aircraft. Similarly, Norris and Gentry (2) captured and placed harnesses and transmitters on gray whale calves, *Eschrichtius robustus*, in a breeding lagoon of Baja California, Mexico.

Table 1. Summary of diving depth and frequency from 608 hours of monitoring at sea.

Fur seal	Body weight estimate (kg)	Recording time (hours)	Number of dives to:							Total dives
			0 to 20 m	21 to 50 m	51 to 80 m	81 to 110 m	111 to 140 m	141 to 170 m	171 to 200 m	
1	35	167	301	314	37	9	13	2		676
2	35	92	1148	1	10	9	49	7		1224
3	35	200	54	478	89	1	4		2	626
4	50	149	201	200	14	7	6	3		431

1). The large number of shallow dives between 0 and 20 m are impossible to interpret without finer resolution in our recorders or some direct observations at sea, or both. Their short duration (< 1 minute) and their clustered occurrence suggest they could be for either shallow feeding or more general diving activity, such as during travel from one place to another.

The deeper dives between 20 and 140 m are probably associated with hunting and feeding. These dives lasted from 2 to 5 minutes, and they were usually clustered as a series of dives, often with striking consistency for depth. For example, seal 2 made 13 dives between 110 and 140 m in a space of 3.6 hours. Each dive lasted from 3.3 to 3.4 minutes, and the interval between dives ranged from 6 to 30 minutes (\bar{X} = 17 minutes).

The deepest dive measured was 190 m, nearly twice that previously reported for this species (7). The duration of this dive (5.4 minutes) was one of the longest recorded. In previous studies in which adult female fur seals were forcibly submerged, their tolerance was only 5 minutes (8).

The diving profile (the plot of depth against time) of the 190-m dive showed that little time was spent at any depth. The average rate of depth change was 70 m/min. This rate is about the same as that of Weddell seals when they are routinely diving to depths of 200 to 400 m (9).

Considerable behavioral, ecological, and physiological information may be gleaned from the two variables, depth and time. Although we have investigated only the northern fur seal, this method of study could be used with many other species of marine mammals.

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5. The recorder was designed and built by J. O. Billups, G. L. Kooyman, and G. V. Sarno. We are currently making certain modifications in it.
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Insulin Binding to Cultured Human Fibroblasts Increases with Normal and Precocious Aging

Abstract. Specific and nonspecific [125 I]insulin binding and concentration of unlabeled hormone producing 50 percent competition with 1.0 nanomolar [125 I]insulin for specific binding sites correlated positively with age of fibroblast donors. Cells from four children with precocious aging—three with progeria and one with Rothmund syndrome—resembled those from the chronologically old.

Cultured human fibroblasts can be used to study genetic-regulatory aspects of insulin binding in normal and abnormal states without ethical restraints. With stable diploid human cells several generations removed from donor neurohumoral influences, the principal experimental variable is considered to be

the genetic endowment of the cell strain (1).

We found positive correlation between age of the fibroblast donor and the amount of insulin, present at 1.0 nM concentration, bound to specific receptor sites. The most significant correlations were between donor age and (i) binding

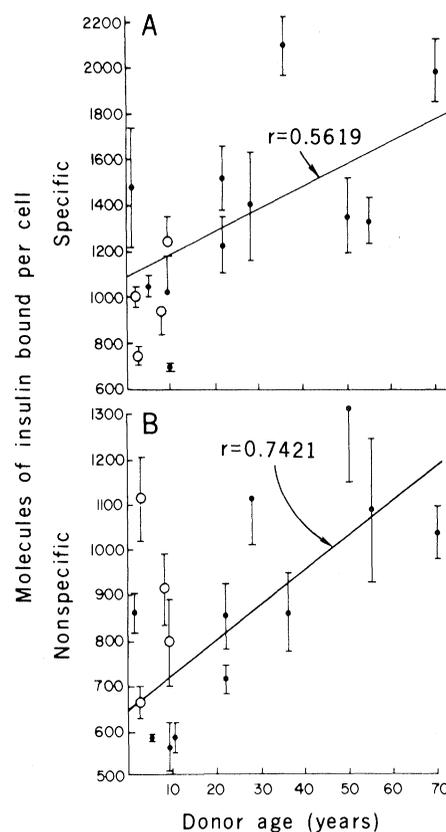


Fig. 1. (A) Correlation between specific binding of radioactive insulin to cultured fibroblasts and age of normal, nondiabetic cell donors ($P < .10$). Midpassage fibroblasts [generation 20 to 45, chosen by known life-span of the strain (12)] were grown to confluence in 100-ml plastic dishes in Eagle's minimum essential medium supplemented with 15 percent fetal calf serum (20). Studies were performed a day after the monolayer reached confluence. All plates were examined for medium pH, confluence, and evidence of abnormality; subcultures were made from two plates to confirm vitality. Growth medium was aspirated from the plate and the cell layer washed twice with 6 ml of buffered saline at room temperature. Eagle's minimum essential medium (1.2 ml) with 1 percent bovine serum albumin buffered with 20 mM Hepes adjusted to pH 7 was added. [125 I]-labeled and native insulin were mixed with media before addition to the dish. After 45 minutes of incubation at room temperature the medium was removed for counting as the free fraction. The cells were scraped from the plate in 1 ml of buffered saline with a silicon-coated rubber scraper. The suspension of dislodged cells was withdrawn into a silicon-coated Pasteur pipette, placed in a plastic microcentrifuge tube, and centrifuged for 2 minutes at 10,000g; the supernatant was discarded and the cell pellet radioactivity was determined (21). [125 I]insulin (50 to 100 μ Ci/ μ g) was prepared from zinc-free pure bovine insulin (Connaught Laboratories) by the method of Hamlin

and Arquilla (22). Material was kept lyophilized at -4°C and assayed for biological activity by the method of Gliemann (23); no loss of biological activity occurred for as long as 8 months under these conditions. Closed circles are normal strains; open circles are strains from precociously aged patients. Each point is the mean of three or more replicate experiments on different days; vertical bars, S.E.M. Specific binding is calculated from the difference between the radioactivity of cell pellets incubated with [125 I]insulin alone and those incubated in the presence of 100-fold native insulin. (B) Correlation between nonspecific insulin binding to cultured fibroblasts and age of normal donor ($P < .01$). Nonspecific binding was that fraction of [125 I]insulin added to the medium that remained bound to cells in the presence of a 100-fold excess of native peptide.