the accuracy of the discharges. Pinching with forceps had shown beetles with replete glands (previously undisturbed for at least 2 weeks) to be capable of discharging upward of 15 times before exhaustion of their reserves. However, in the tests with ants the beetles were only rarely forced to discharge in rapid succession, since each ejection was followed by a period of relative invulnerability during which approaching ants were apparently repelled by residual secretion remaining on the beetle from the previous discharge. Benzoquinone vapors are potently deterrent to ants, and protracted postdischarge invulnerability had also been noted in tests with brachinines (*8*).

Goniotropis and *Ozaena* belong to the Ozaenini, one of two major subdivisions of the Paussinae. The other subdivision, the Paussini, includes species highly specialized for life in ant colonies (*2*). Although they too discharge benzoquinones (*3, 4*), no one knows how their defenses figure in their interactions with enemy or host. But they too have elytral flanges, which we presume to be functionally analogous to those of the Ozaenini.

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7. The technique involved causing the beetles to discharge on precalibrated heat-sensing devices (thermocouples and microthermistors), as previously described for brachinine bombardiers (*5*). The temperature of the *Goniotropis* spray was 65° ± 5°C (N = 10) with a maximum of 81°C.
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9. Adult paussines are insectivorous; we maintained them for months in the laboratory on pieces of freshly killed mealworms (larvae of *Tenebrio molitor*).
10. The specifications of the paper are given elsewhere (*8*).
11. Omitted from Fig. 1 is an indication of the splatter that often occurred in forward discharges (particularly in the more massive first discharges of beetles with replete glands, as in Fig. 2A). This splatter was a consequence of (i) spontaneous breakup of the jet of fluid as it shot ahead beyond the length of the beetle, and (ii) impact of the jet with legs, antennae, and (when the beetle's body was not held straight) the margins of head and thorax. There was also usually some splatter from the gland opening itself.
12. Flange removal stopped just short of the margin of the elytron.
13. Each beetle was seized by its respective leg and, while discharging (the discharges are faintly audible and can be spotted by the appearance of brownish secretion on targeted appendages), was killed by abrupt immersion in chilled (−195°C) liquid Freon. It was then transferred while still frozen to the precooled stage of a tissue freeze-drier for desiccation and given a conventional metallic coating for electron microscopic examination.
14. *Goniotropis nicaraguensis* is the only species of those listed in our previous chemical papers (*3*) that has the hairs.
15. A scatter of microdroplets from the hairs would not have been resolved at the magnification of our motion pictures; only coarse scatter from other body parts was detectable (*11*).
16. The hairs may also provide sensory feedback from the spray; however, they are rigidly attached at the base and are not movably inserted into sockets as mechanoreceptors usually are.
17. Duration was calculated from counts of consecutive film frames in which individual discharges remained visible, and velocity by measurement of the distance advanced by the leading edge of the jet of spray in consecutive frames.
18. For the ballistic calculation, beetles fastened to rods were adjusted in horizontal stance (direction of emission of spray from flange is then also horizontal) on sheets of indicator paper and caused to discharge forward in response to pinching of forelegs. Spray velocity (v) was calculated from $v = D/\sqrt{2d/g}$, where D is distance from flange to remotest point ahead of beetle where paper was marked by secretion; d is height of flange above paper, and g is acceleration due to gravity.
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Play Behavior: Persistence, Decrease, and Energetic Compensation During Food Shortage in Deer Fawns

Abstract. *White-tailed deer fawns continued to play despite an experimentally induced 33 percent milk shortage. They reduced play by 35 percent and general activity by 9 percent but increased grazing by 62 percent, resulting in virtually complete energetic compensation. This demonstrates the importance of play behavior in a mammal's activity budget.*

The bioenergetic priority of play behavior in immature mammals has been a matter of dispute ever since Spencer (*1*) postulated "energy surplus" as the proximate source of play. Since long-term effects of play (or its absence) are difficult to measure, the magnitude of the selective advantage (but not its specific nature) of play could be estimated by how much the growing organism is willing to pay for being able to play, especially under energetically adverse conditions. Will play be dropped for short periods and without ill effects? Is it a built-in reserve activity, "behavioral fat" (*2*)?

We report the redistribution of energy allocated to various activities by young mammals when receiving a reduced amount of milk. They continued to play, but at a lower rate, which was proportional to the food reduction. They spent more time resting but increased their grazing drastically. These adjustments resulted in only minor changes of both the energetic input and output.

Play behavior comprises vigorous activities, such as leaping, running, chasing, striking, or wrestling, in the absence of an immediate need for fleeing or fighting. It can be solitary, parallel (that is, socially facilitated without interaction), or social (that is, interactive). Many adaptive functions have been postulated for the play behavior of young mammals. They range from general neuromuscular and cardiovascular exercise, to sensory stimulation of the developing nervous system, to learning about the environment, and to development of social, reproductive, and maternal skills. About 30 such functions have been attributed to play and exploration (*3*).

The energy budget of the young animal covers expenditures for maintenance (food getting, shelter seeking, predator avoidance, and thermoregulation), growth, and play behavior (*4*). Primates play less during periods of food shortage. Rhesus monkeys (*Macaca mulatta*) on Cayo Santiago Island played 17 times less during a 15-day food shortage. All behaviors except foraging decreased in frequency, and six of the 69 animals died, indicating the severity of the lack of food (*5*). No play occurred in two troops of 23 and 27 squirrel monkeys (*Saimiri*) in a southwestern Panama forest during 261 hours of observation. There was little of the monkeys' preferred foods available, and foraging took up 95 percent of their waking time. During the remaining 5 percent of the time, there were many social situations that in other squirrel monkey populations would have led to social play (*6*). Captive squirrel monkeys forced to obtain powdered food out of a container played as little as 1 percent of normal (*7*). There was, however, no complete cessation of play. When their food ration was

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Table 1. Play running differences between siblings during experimental and control periods. During control periods the individuals within each litter did not differ in their running play. The pairwise *t*-test for litter A during the control periods compared one individual with the average of the other two. N.S., not significant.

Litter	Running: difference		Analysis of variance		Paired *t*-test	
	Distance (m)	Percentage	*F*	*P*	*t*	*P*
	Two experimental periods (N = 10 days)					
A	−407 ± 169.3	−43.2 ± 11.8	8.392	< .025	2.404	< .05
B	−176.4 ± 30.5	−31 ± 5.3	33.466	< .001	5.848	< .001
	Three control periods (N = 15 days)					
A	−3.0 ± 40.1	+8.0 ± 13.2	0.109	N.S.	1.17	N.S.
B	+48.8 ± 27.5	+12.4 ± 10.6	0.637	N.S.	1.05	N.S.

reduced by 50 percent, a "complete drop-out of play" resulted (*8*).

In free-ranging ungulates, the frequency of play behavior may vary with the quality of the environment, especially the food supply. Mountain sheep play less in poorer habitats (*9*). Lambs of bighorn sheep (*Ovis canadensis*) play more in mountains than in the desert. This has been attributed to more hazardous terrain (such as cacti) in the desert (*10*).

To study the effect of food shortage on play, the ratio of activity to rest, and compensatory grazing, we reduced the milk supply of white-tailed deer fawns (*Odocoileus virginianus*) and recorded these behaviors before, during, and after the reduced intake.

Five female fawns (one set of triplets and one pair of twins, and 2 days apart in age) born in captivity were observed from their 11th day of life. This sample size is hard to surpass with large wild animals. They were kept as one group in an indoor room (4 by 2.5 m) with an outdoor pen (4 by 10 m), were given free access to Jersey cow milk (three times per day), and were able to graze.

Starting at the age of 27 and 29 days respectively, one fawn of each litter for 5 days received only two-thirds of the amount of milk (1156 ± 92 and 1399 ± 111 ml, respectively) it had consumed on the average during the preceding 5 days. After the animals drank the milk, water from a bottle was freely available, a procedure allowing the same amount of sucking and more or less the same stomach tension as when undeprived. The animals actually consumed 1956 ± 130 and 1985 ± 69 ml of fluid, respectively. Two fawns of litter A and one from litter B were controls. After the milk deprivation, the fawns were allowed milk at all times for 5 days, and then the milk supply for the remaining three fawns was reduced to two-thirds of their prior 5-day average consumption of 1418 ± 85, 1616 ± 52, and 1516 ± 103 ml, respectively). They drank 2518 ± 228, 2629 ± 164, and 1732 ± 92 ml of fluid (milk plus water) daily during the deprivation period. Finally, all fawns were given free access for a 5-day postexperimental control period. In addition to measuring milk consumption, we recorded all play, as well as the animals' activity—such as walking, grazing, or resting every 60 seconds. Running speed was timed with a stopwatch; all other play patterns were counted. The fawns were observed from the time of their release from the indoor room (0719 ± 4 minutes) to the end of their morning activity bout (all animals reclined; 0835 ± 3 minutes). Observation time averaged 76 ± 4 min/day, from 0719 ± 4 minutes to 0835 ± 3 minutes.

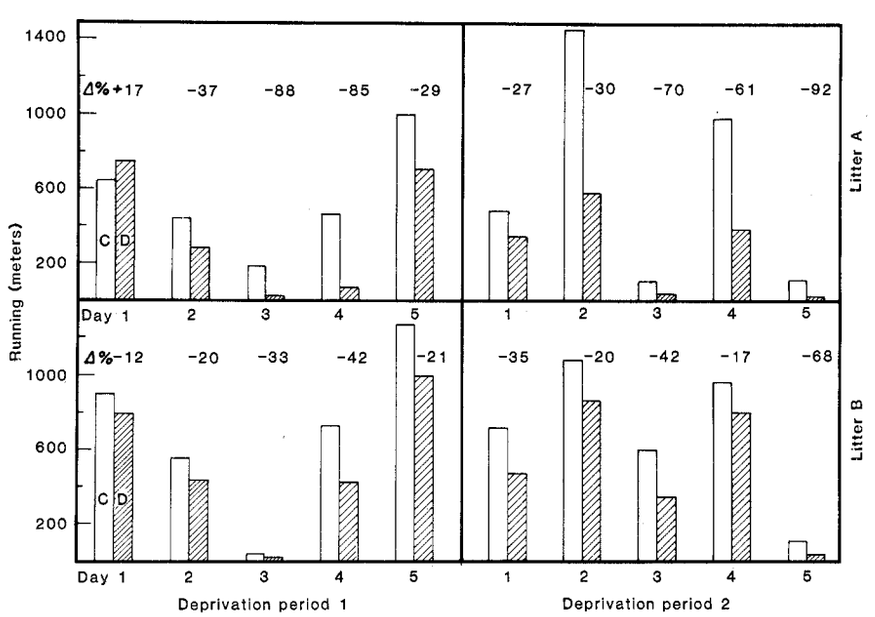

Fig. 1. Comparison of play running per day (in meters, left ordinate) by partially deprived fawns (*D*) with that of controls (*C*). (Top) Litter A; (bottom) litter B. (Abscissa) Days (1 to 5) of the two deprivation periods, which were 5 days apart. The percentages represent the difference in the amount of play between deprived and control animals.

The daily play bout occurred between 0730 and 0830 (peak between 0744 ± 6.9 minutes and 0831 ± 6.4 minutes). The distinct motor patterns were head jerk, running at 4 meters per second, leaping, bucking, butting, mounting, and sudden reclining (*11*). Running constitutes 91.5 percent of play in terms of estimated energy spent. Therefore, running was used to measure play (number of 20-m laps).

Pairwise, day-by-day comparison of the amount of running play of deprived and control fawns shows a significant drop of play during milk deprivation (Fig. 1). The overall average drop was 34.9 ± 6.4 percent (standard error of the mean). During the three control periods of 5 days each (which occurred before, between, and after the experimental periods), the amount of play did not differ between individuals in each litter (Table 1).

Deprived fawns were 9.3 ± 3.5 percent less active than before deprivation and 13.5 percent less active than simultaneous sibling controls ($F = 9.516$; $P < .01$).

Grazing increased by 62.4 ± 17.8 percent ($t = 17.27$; $P < .001$). Deprived individuals grazed 46.1 ± 13 percent more than simultaneous sibling controls ($F = 5.466$; $P < .05$).

Thus, adjustments were made in all activities measured. (Growth was not included because of the short-term nature of the experiment.) Before deprivation, each of the five fawns on the average obtained 1177 ± 52 kcal daily from their 1421 ± 77 ml of milk (*12*) and an estimated 589 ± 26 kcal by grazing (*13*).

The 62.4 percent increase in grazing yields an estimated 368 additional kcal, almost compensating for the loss of 392 kcal (one-third of 1177 kcal) as milk. The total input during deprivation was 1746 kcal/day (789 as milk, 589 + 368 from grazing), or 98.9 percent of normal. On the output side, an estimated 483 kcal/day are needed for basal metabolism plus 22 kcal/day (before deprivation) for standing, walking, running, play, and miscellaneous, such as ruminating (*11*) (total, 505 kcal/day) (*4*). During deprivation the 9 percent activity decrease saves 1.2 kcal/day and the 35 percent play decrease saves 1.9 kcal/day, but the increased grazing costs 5.4 kcal. The total output changes from 505 to 507.3 kcal/day, or by only 0.5 percent. Thus, the small input (−1.9 percent) and output (−0.5 percent) changes represent an almost complete energetic compensation in the face of a 33 percent milk reduction.

Output compensation has been demonstrated earlier: Black-tailed deer fawns are more active (nonplay) after experimental play deprivation (*14*) and, conversely, show less play running if they are forced to increase walking prior to their play bout (*15*).

The 35 percent drop in running play seems proportional to the 33 percent milk decrease. Although energy required for running play constitutes only 0.9 percent of the estimated daily energy output (4.5 of 505 kcal), exclusive of growth (or about 20 percent of the activity budget), play was not dropped completely. Instead, adjustments were made "across the board" for all behaviors monitored. Unlike primates (*16*), deer play very little and thus may derive developmental benefits from play at very low cost.

Our experiment supports the notion that play can function as "behavioral fat" during a temporary noncatastrophic food shortage. It is reduced, while at the same time other behaviors such as grazing can still be substantially increased. The persistence of play, on the other hand, points to the importance of play.

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Detection of Mutagens in Complex Samples by the *Salmonella* Assay Applied Directly on Thin-Layer Chromatography Plates

Abstract. *A new technique is reported in which components in complex samples are separated on thin-layer chromatography plates and their mutagenic effect is registered directly on the plates by means of the* Salmonella *assay. The method is quick and simple and particularly useful for screening large numbers of environmental samples. Qualitative comparisons of mutagens in different samples can easily be made. Registered mutagens can be identified by the chemical analysis of extracts from duplicate plates.*

One of the major goals in environmental chemistry is to evaluate potentially hazardous compounds in the process streams, effluents, and other parts of the environment. The traditional way of characterizing a complex mixture has been by chemical analysis of groups of compounds or individual compounds known to be hazardous. When these methods are used, unknown carcinogens or mutagens may remain undetected. Improved strategies rely on chemical or physical fractionations and subsequent mutagenicity testing of the individual fractions. Such methods, however, require very detailed analysis and can easily become very expensive. Furthermore, they have drawbacks such as sample dilution, reactions of compounds through the fractionation procedure, and irreversible adsorption of biologically active compounds onto column materials or equipment.

The use of thin-layer chromatography (TLC) to separate complex samples and the testing of the separated components

Table 1. Mutagenicity testing of known mutagens by the Ames TLC technique. The compounds were chromatographed on silica TLC plates. The number of revertants around the test spot (lower left section of Fig. 1) was scored as follows: +++, > 40; 40 > ++ < 20; +, < 20; −, not detectable. Solvent system Ia was chloroform; solvent system II consisted of chloroform, benzene, ethyl acetate, methanol, and aqueous ammonia (4 : 1 : 3 : 2 : 0.2).

Solvent system	Amount spotted on (μg)	Revertants per plate			
		T98		TA100	
		With S-9	Without S-9	With S-9	Without S-9
		2-Aminoanthracene			
II	1	++	−	+	−
II	5	+++	−	+	−
II	10	+++	−	+++	−
		1-Nitropyrene			
Ia	0.5	++	+++	+	+
Ia	1	+++	+++	+	+
		2,4-Diaminoanisole			
II	50	++	+	−	−
II	100	+++	+	−	−
		Benz[a]*anthracene*			
Ia	5	−		−	
Ia	10	−		−	
Ia	20	−		−	

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