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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References and Notes

1. H. Spencer, *The Principles of Psychology* (Appleton, London, 1878).
2. D. Muller-Schwarze, in *Evolution of Play Behavior*, D. Muller-Schwarze, Ed. (Dowden, Hutchinson, and Ross, Stroudsburg, Pa., 1978), p. 382.
3. J. D. Baldwin and J. I. Baldwin, in *Primate Bio-Social Development: Biological, Social and Ecological Determinants*, S. Chevalier-Skolnikoff and F. E. Poirier, Eds. (Garland, New York, 1977), pp. 343–406.
4. K. L. Blaxter, *The Energy Metabolism of Ruminants* (Hutchinson Scientific and Technical, London, 1967); D. M. Walker and G. J. Faichney, *Br. J. Nutr.* **18**, 187 (1964).
5. J. Loy, *Am. J. Phys. Anthropol.* **33**, 263 (1970).
6. J. D. Baldwin and J. I. Baldwin, *Folia Primatol.* **18**, 161 (1972).
7. ———, *Z. Tierpsychol.* **40**, 1 (1976).
8. C. H. Southwick, *Behaviour* **28**, 1982 (1967).
9. V. Geist, *Mountain Sheep* (Univ. of Chicago Press, Chicago, 1971); D. M. Shackleton, thesis, University of Calgary (1973); B. L. Horejsi, thesis, University of Calgary (1976).
10. J. Berger, *J. Zool.* **188**, 251 (1979).
11. Essentially the same motor patterns as described for the black-tailed deer (*O. hemionus columbianus*) [D. Muller-Schwarze, *Behaviour* **31**, 144 (1968)].
12. Jersey milk contains 4.2 percent protein, 5.2 percent fat, and 4.8 percent carbohydrates. The available energy is 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for carbohydrates [L. E. Harris, "Biological Energy Relationships and Glossary of Energy Terms," *NASNRC Publ.* (1966), No. 1411]. Thus, 100 g of Jersey milk contains 82.8 kcal of physiologically available energy.
13. One-month-old fawns of the white-tailed deer obtain about one-third of their diet by grazing [A. Moen, *Wildlife Ecology* (Freeman, San Francisco, Calif., 1973)].
14. D. Muller-Schwarze, in (*11*).
15. ——— and C. Muller-Schwarze, *Bonn. Zool. Beitr.* **20**, 282 (1969).
16. E. O. Smith, in *Social Play in Primates*, E. O. Smith, Ed. (Academic Press, New York, 1978), p. IX.

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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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in the Ames *Salmonella* test have been reported by others. In studies of atmospheric reactions Pitts *et al.* (*1*) used TLC to separate the components, and biological activity was determined by mutagenicity testing of the extracted spots. Hayashida *et al.* (*2*) carried out a TLC separation of a urine sample, sectioned the chromatography plate, and tested the sections individually for mutagenicity in a spot test. Likewise Wilson *et al.* (*3*) and Issaq *et al.* (*4*) separated mutagens from complex mixtures on TLC plates and tested extracts in the *Salmonella* assay. By combining TLC with the Ames *Salmonella* assay, one may detect mutagenic chemicals by using bacteria as selective detectors of mutagenic compounds directly on the TLC plates. The number of microbiological tests needed to characterize a sample is greatly reduced. With this method, mutagenic compounds in the samples can be immediately detected and their localization on the plate gives an indication of the chemical properties. For the identification of mutagenic compounds, duplicate TLC plates may be developed; areas containing mutagens may be scraped off and analyzed with state-of-the-art methods. The technique is simple and has a large potential for routine testing and for qualitative comparisons of complex mixtures.

We used commercially available silica, cellulose, and reversed-phase TLC plates (10 by 10 cm), developed in absolute ethanol and dried for 5 minutes at room temperature and then for 20 minutes at 80°C. The plates were subsequently treated as sterile. No mutagenic areas were observed on blank TLC plates, only an expected number of spontaneous revertants.

Samples (1 to 3 μl in acetone) were spotted on the plates, and the separation was carried out at room temperature in a 12- by 12-cm chamber. Components were made visible in fluorescent light at 254 and 350 nm or were made visible on parallel plates by conventional techniques (*5*).

We carried out the mutagenicity assay essentially as described by Ames *et al.* (*6*), using *Salmonella* strains TA98 and TA100 with and without S-9 mix prepared from Aroclor 1254–induced rat liver, except that the tests were performed directly on the developed TLC plates. We prepared minimal agar plates by placing the developed TLC plates in the bottom of 14.5-cm petri dishes and carefully adding 30 ml of minimal agar to each plate. After 1 to 2 hours, the test strain (0.3 ml), with or without S-9 mix (1.5 ml), was added to 6 ml of top agar, mixed, and poured on top of the minimal agar. After 3 days of incubation at 37°C, clusters of histidine revertant colonies were observed in areas containing mutagenic compounds on the TLC plate. Several combinations of layers were tried out, and the sequence of TLC plate, minimal agar, and top agar was adopted as optimal. To generate a mutagenic effect by this technique, the compound must reach the cell while growth factors (in particular, histidine) and enzymes in the S-9 mix are still available. The release of mutagens from the TLC layer and their diffusion into the agar requires time, and it is important to control this parameter. The timing is influenced by the diffusion behavior of individual mutagens and by the initial levels of growth factors and cell density.

Special attention was given to the diffusion of mutagens in the agar layer. Different model compounds were chosen, including mutagens such as sodium azide (polar and water-soluble), 2-aminoanthracene (2-AA, intermediate polarity), and benzo[*a*]pyrene (BaP, nonpolar), all of which are known mutagens in the *Salmonella* assay (*7*). The test compounds were spotted on the TLC plates without any chromatography. Sodium azide, a direct-acting alkylating agent, was tested in strain TA100 without S-9. As the compound diffused over the plate, revertant colonies were scored all over the plate and not in any one distinct area. The compound 2-AA gave a clear mutagenic effect in both strains with the addition of S-9. A dose response was observed with a detection limit of 1 and 0.05 μg for strains TA100 and TA98, respectively. The compound BaP, a mutagen needing metabolic activation, was tested in both strains in the presence of S-9. No mutagenicity was observed. This problem has been pointed out by Ames *et al.* (*6*), who found that most polycyclic aromatic hydrocarbons (PAH) do not give a mutagenic response in a spot test. The addition of 2 percent

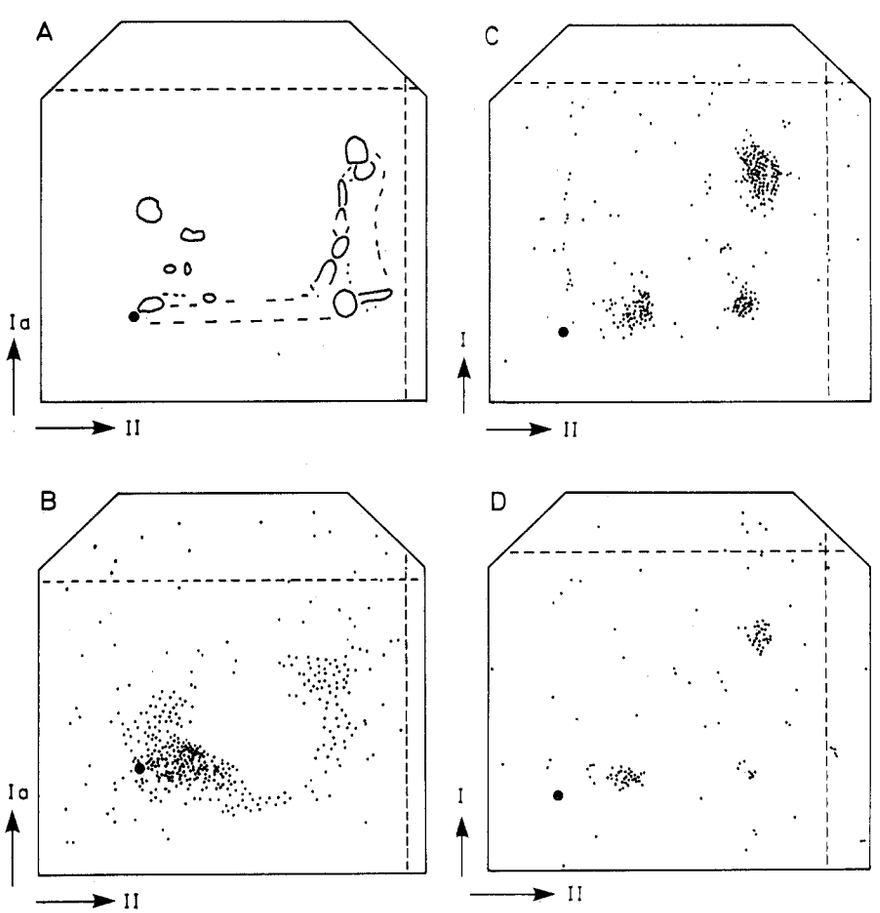

Fig. 1. Application of the Ames TLC technique with the use of strain TA98 to extracts from an air sample and a typewriter ribbon. The control plate containing approximately 60 revertants evenly distributed on the plate is not shown; ●, application point; – – –, solvent front. Extract of an air sample from St. Louis, Missouri: (A) registered visual spots and spots visible in ultraviolet light; (B) registered revertants on the plate in the absence of S-9. Extract of typewriter ribbon: (C) registered revertants on the plate in the absence of S-9; (D) registered revertants on the plate in the presence of S-9. Solvent systems Ia and II are described in Table 1. Solvent system I consisted of chloroform and acetic acid (10:0.1).

surfactants or solvent (dimethyl sulfoxide) to the minimal agar did not influence the solubility of BaP. Accordingly, we examined mutagenic compounds of intermediate polarity in further studies of the *Salmonella*-TLC system.

Results of mutagenicity testing of chemicals chromatographed on silica TLC plates with two different solvent systems are given in Table 1. (With 2-AA, no difference was found when cellulose plates were used instead of silica plates.) Using 1-nitropyrene, a direct-acting mutagen in the Ames test (*8, 9*), we observed a strong mutagenic effect with strain TA98 both in the presence and in the absence of S-9. Because TA100 is not as sensitive as TA98 to the mutagenic effects of nitropyrenes (*8*), the number of revertants that we observed with TA100 decreased accordingly.

The hair-dye component 2,4-diaminoanisole (2,4-DAA) is a mutagen that requires metabolic activation in the *Salmonella* assay (*7*). The compound was clearly mutagenic in strain TA98 in the pesence of S-9 in one-dimensional TLC. In addition to the 2,4-DAA area, three other mutagenic areas were observed on the plate. These areas, probably due to impurities, demonstrate the usefulness of the method to test the purity of reference compounds and the biological effect of impurities.

One area where the Ames TLC technique will find application is on extracts from environmental samples. A major problem in the mutagenicity testing of complex mixtures is toxicity, for example, in the testing of emission samples from various combustion sources. If the extracts are toxic, mutagenic activity in the Ames test may not be observed unless a chemical fractionation is performed. With this new technique, the components of the mixture are separated on the TLC plate before mutagenicity testing. Toxic compounds may then be separated from the mutagenic compounds and thereby not interfere with the mutagenicity test.

Air pollutants include a large number of organic compounds, and the mutagenicity of airborne particles has recently been reviewed by Chrisp and Fisher (*10*). We have applied the Ames TLC technique to extracts of airborne particulate matter from St. Louis, Missouri. The results are shown in Fig. 1, A and B. Figure 1A indicates the spots visualized at 254 and 350 mm. Figure 1B shows the revertants observed in strain TA98 without S-9. There was a clear increase in the number of revertants in a zone near the application point as well as a zone with R_F values somewhat lower than those found for normal PAH compounds (indicating more polar compounds). In the middle of the plate, there was an area with no revertants, in which the background lawn of bacteria was inhibited as a result of toxic compounds in the sample. The addition of S-9 reduced the number of revertants observed on the plate. Comparable results were obtained with strain TA100, but the number of revertants was lower.

Mutagens associated with photocopy toners and typewriter ribbons were recently described by Löfroth *et al.* (*8*), Rosenkranz *et al.* (*9*), and Alfheim *et al.* (*11*). In our investigation extracts of correctable film ribbons for typewriters were spotted on TLC plates, chromatographed, and tested in the *Salmonella* assay. Clusters of colonies were observed in three main zones with TA98 in the absence of S-9: one cluster near the application point, another to the right top (that is, high R_{FI} and high R_{FII}), and a third cluster in the bottom right corner (low R_{FI} and high R_{FII}) (Fig. 1C). In the presence of S-9, the number of revertants was reduced (Fig. 1D).

Our results demonstrate that the direct detection of separated mutagens on TLC plates is possible. Mutagens are detected as clusters of histidine revertant colonies in agar gels placed on top of the plates. Several mutagenic areas are detectable on each plate. The chemical and physical properties of the mutagens may be inferred from their TLC behavior. Mutagens may also be isolated from replicate TLC plates and identified by chemical analysis. Because the compounds are separated, effects arising from toxic substances in the sample may, in principle, be avoided. With the Ames TLC technique, it is possible to investigate amounts of individual compounds from a few nanograms to about 100 μg. For compounds of intermediate polarity, the sensitivity of the test approximates the conventional Ames *Salmonella* test.

This new test, which is quick and easy to perform, may be applied to a variety of samples, such as extracts of air samples or pure chemicals. As compounds are accessible on the TLC plate, reactions may be carried out to study the potential formation of new mutagens or the destruction of existing ones. For example, one might study the effects of exposure to radiation, atmospheric NO_x or SO_2 and SO_3, or enzymic degradation. So far, the best results have been obtained with substances of intermediate polarity (*12*).

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References and Notes

1. J. N. Pitts, Jr., K. A. Van Cauwenberghe, D. Grosjean, J. P. Schmid, D. R. Fitz, W. L. Belser, Jr., G. B. Knudson, P. M. Hynds, *Science* **202**, 515 (1978).
2. S. Hayashida, C. Y. Wang, G. T. Bryan, *Gann* **67**, 617 (1976).
3. B. W. Wilson, R. Pelroy, J. T. Cresto, *Mutat. Res.* **79**, 193 (1980).
4. H. J. Issaq, A. W. Andrews, G. M. Janini, E. W. Barr, *J. Liq. Chromatogr.* **2**, 319 (1979).
5. D. C. Fenimore and C. M. Davis, *Anal. Chem.* **53**, 252A (1981).
6. B. N. Ames, J. McCann, E. Yamasaki, *Mutat. Res.* **31**, 347 (1957).
7. J. McCann, E. Choi, E. Yamasaki, B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5135 (1975).
8. G. Löfroth, E. Hefner, I. Alfheim, M. Møller, *Science* **209**, 1037 (1980).
9. H. S. Rosenkranz, E. C. McCoy, D. R. Sanders, M. Butler, D. K. Kiriazides, R. Mermelstein, *ibid.*, p. 1039.
10. C. E. Chrisp and G. L. Fisher, *Mutat. Res.* **76**, 143 (1980).
11. I. Alfheim, M. Møller, G. Löfroth, L. Nilsson, paper presented at the 12th annual meeting of the Environmental Mutagen Society, San Diego, Calif., 5 to 8 March 1981.
12. Work is in progress in our laboratory to increase the sensitivity of the test to compounds that are more nonpolar.
13. This work was supported in part by the Electric Power Research Institute, Palo Alto, Calif.

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