however, this is accompanied by an increase in the wrong verdicts "not significant" when there is a real difference between the effects of the treatments.

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References and Notes

1. I am greatly indebted to Donald Mainland for suggesting the method, for his constructive criticisms and suggestions, and for his interest and encouragement. I am indebted to both Mainland and Ruth M. Smith for carrying out the sampling experiments. For random allocation methods also see A. L. Edwards, *Experimental Design in Psychological Research* (Rinehart, New York, 1951), p. 23.
2. R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agricultural and Medical Research* (Oliver and Boyd, London, ed. 3, 1948), p. 104.
3. W. Feller, *An Introduction to Probability Theory and its Applications* (Wiley, New York, 1950), pp. 78–81 and 217–218.
4. R. A. Fisher, *Statistical Methods for Research Workers* (Hafner, New York, ed. 11, 1950), p. 96.
5. D. Mainland, *Can. J. Research* E26, 1 (1948).

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Excretion Patterns of Rats Following Total-Body Exposure to X-radiation

Nitrogen and sulfur metabolism change radically following total-body exposure to ionizing radiation, with excretion increasing within 1 day and remaining high in nonsurvivors but decreasing after a period in survivors (*1–5*). Detection of early metabolic changes could be of vital importance in a national emergency (*6*).

Twelve young (180 ± 5g) male Sherman-strain rats, after a 3-hour fast, were irradiated in lucite chambers with 550 r (LD_{42}^{30}) delivered from a deep-therapy G.E. Maximar X-ray machine (*7*). Animals were maintained at 25°C and were fed Purina Lab Chow and water *ad libidum*. Complete individual 24-hour fasting (water supplied) urine samples were collected before and at intervals after irradiation. Thirteen compounds per sample were determined in triplicate using paper chromatographic and colorimetric methods (Table 1). The individual means were averaged by groups surviving 30 (*5*), 14 (*2*), and 11 (*3*) days, groups I, II, and III, respectively. Each animal served as its own control owing to the wide individual variation, and all percentage changes were related back to the preirradiation-sample values of each group.

Urine volumes and the excretion of most compounds became elevated in all groups. Glycine was subnormal throughout the postirradiation period, while taurine, valine, and aspartic acid were, for the most part, subnormal after the first day. Urea and alanine were elevated during the entire period. One-day postirradiation phosphate, taurine, and alanine increased, while histidine and aspartic acid decreased progressively from groups I to II to III, and the uric acids of the nonsurvivors were significantly greater than those of group I. On day 5, alanine and glycine trends remained unchanged, while group III diverged further from groups I and II by maintaining an elevated phosphate, uric acid, and creatinine and a depressed histidine. Glutamic acid and aspartic acid dropped in all.

During the acute phase, when animals were dying (days 10 to 14), urine volumes, phosphate, urea, and alanine were elevated, while taurine and glycine were depressed. The divergence between groups I and II and group III was marked on day 9 with respect to everything except glycine, valine, and aspartic acid. Animals dying on day 14 (group II) were markedly different from those in group I in everything except phosphate, glycine, alanine, and taurine, which were now at about the same levels as the premortal values of group III. Creatinine and aspartic acid were depressed in group II, while group I had returned to normal levels. Uric acid increased in both. Histidine dropped in group II to the premortal levels of group III, while it steadily increased in group I. Values of groups II and III just prior to death were nearly the same for some compounds but were significantly different for others. It is apparent that some of these changes are common precursors of impending death,

Table 1. Relative percentage changes in excretion patterns of rats exposed to a total-body dose of 550 r x-radiation

Compound determined	Survival group	Post irradiation days							
		−3	1	5	9	13	17	23	29
Phosphate	I	100	163†	83	144	201†	150	121	136
	II	100	209	64	141	208			
	III	100*§	212*†	160	214*†‡				
Creatinine	I	100	112	84	93	115	128†	107	130
	II	100	101	88	119	72*			
	III	100*§	197*†	134*	135*‡				
Urea	I	100	127	101	111	140†	149†	113	142†
	II	100	224*	139	143	161			
	III	100*	114*	144	119*‡				
Uric acid	I	100	242†	85	72	115	130	124	126
	II	100	289*†	88	63	102			
	III	100*§	288*†	164	237†				
Taurine	I	100	118†	92	60†	67†	67†	76†	96
	II	100	147*†	97	81†	69			
	III	100	162*†	80*†	66*†				
Glycine	I	100	84	67†	57†	69†	66†	71†	70†
	II	100	71†	68†	58†	71†			
	III	100	81	68	63†‡				
Valine	I	100	119†	95†	86	86†	86	86	90
	II	100	124*†	118	106	100			
	III	100	122†	94	83				
Alanine	I	100	127	155†	127	109	118	109	145†
	II	100	138	200	107	100			
	III	100	150	200	100*‡				
Aspartic acid	I	100	106	62†	62†	94	75	81	94
	II	100	94	56	62	56			
	III	100	58*†	45*†	64				
Glutamic acid	I	100	77†	74†	97†	116†	139	149	129
	II	100	86	79	90	103			
	III	100§	55*†	60*†	84*				
Histidine	I	100	97	105	122	132†	136†	226†	239†
	II	100	71	84	96	79			
	III	100	58	65	70				
Urine volumes	I	100	201†	156	123	132	123	114	103
	II	100	378†	219†	186†	186†			
	III	100*§	221†	352	182*†‡				
Body weight	I	96‖	95	85†	79†	81†	82†	87†	92
	II	96‖	95	86†	78†	78†			
	III	96‖	95	84†	78†				

Significantly different from the following at $p < 0.05$ (*9*):
* From group I on the same day. † From day 0 within the group.
‡ Final day of group III from final day of group II.
§ From group II on the same day, calculated for day 0 only.
‖ Due to the 24-hour fast.

while others are more dependent upon the physiological state existing during the phase of the radiation-sickness syndrome in which death occurs. By day 29 only taurine and urine volumes had returned to preirradiation levels in group I. The relationship of some of these changes to known sequelae of the syndrome are evident: for example, taurine to depressed —SH (*8*) and disrupted cysteine metabolism, histidine to hemopoiesis, phosphate and uric acid to nucleic acid metabolism, and urea and creatinine to tissue damage and starvation. The other amino acid patterns are difficult to understand at present.

The biological action spectrum of ionizing radiation is very broad—the amount required to inactivate enzyme molecules is greater by several orders of magnitude than that required to kill mammals, and this in turn is much greater than that required to inactivate lymphocytes. However, the difference between the amount of radiation that is needed to give mammals either a 100- or 0-percent survival in a given period is very small. At a given dosage in this range the distinction between survival and death is very fine. A group of animals (inbred or not) irradiated with an identical dose probably vary widely at the time in relative sensitivities and recovery potentialities, owing to both genetic heterogeneity and phenotypic variability. Some individuals survive and some die. The metabolic differences among them are detectable within 24 hours after exposure. Indeed, in this case, short-term survivors had the lowest individual preirradiation values of the 12 rats in urine volumes, phosphate, creatinine, urea, and uric acid. Further study may make possible the construction of an index of survival; for example, on day 1, animals surviving only 10 days had the highest phosphate, taurine, creatinine, and alanine, and the lowest urea, aspartic acid, glutamic acid, and histidine. Taurine is especially interesting because of the small individual variability.

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References and Notes

1. C. Gros and P. Mandel, *Compt. rend.* 231, 631 (1950).
2. G. E. Gustafson and S. Koletsky, *Am. J. Physiol.* 171, 319 (1952).
3. A. M. Brues, *Nucl. Sci. Abstr.* 7, 7 (1953).
4. R. E. Kay and C. Entenman, *Federation Proc.* 13, 520 (1954); R. H. Oster and W. T. Salter, *Am. J. Cancer* 32, 422 (1938); L. H. Hempelmann, H. Lisco, J. G. Hoffman, *Ann. Internal Med.* 36, 279 (1952).
5. E. J. Katz and R. J. Hasterlik, *J. Natl. Cancer Inst.* 15, 1085 (1955).
6. Supported in part by a contract with the Atomic Energy Commission.
7. Radiation factors: 200 kvp, 20 ma, 1.0 mm Al, and 0.9 cm Cu; 70 cm target-center of body distance, 25 cm square field; 47.8 r/min, dose rate.
8. B. Shacter, H. Supplee, C. Entenman, *Am. J. Physiol.* 169, 499, 508 (1952).
9. Because of the large variance within the groups, the Mann-Whitney "U" test [*Ann. Math. Statist.* 18, 50 (1947)] was used except for the low variance taurine and histidine, where Student's "t" test was employed.

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Reward Schedules and Behavior Maintained by Intracranial Self-Stimulation

Olds and Milner have demonstrated a rewarding effect produced by electric stimulation of some areas of the brain (*1*). Rats that could electrically stimulate themselves in the septal region and certain other areas each time they pressed a lever (continuous reinforcement) were able to maintain high lever-pressing rates without any other reward. The present study was undertaken to develop, through the use of reward (reinforcement) scheduling techniques, stable, long-term lever-pressing rates sensitive to the effects of relevant variables.

In contrast to the continuous reinforcement procedure in which every lever press produces the reward, the reinforcement may be programmed in such a way that only occasional responses are rewarded. This may be accomplished by means of a variable-interval schedule, in which the lever is primed to deliver the reward on a random time basis, or by means of a fixed-ratio schedule, in which a fixed number of responses is required to produce the reward. Such schedules have been demonstrated to generate characteristic types of behavior when conventional rewards—for example, food or water—are used (*2*).

A pulse-pair generator recently described by Lilly and his coworkers (*3*) served as the electric stimulus source. Stable lever-pressing rates have been maintained by rats and cats on reinforcement schedules over periods as long as 6 months without any change in the stimulus parameters. The stimulus, delivered through chronically implanted electrodes (*4*), had a frequency of 100 cy/sec and a pulse-pair duration of 0.1 msec, with amperage varying from animal to animal. The duration of each train of pulse-pairs was 0.5 sec, regardless of the duration of the lever-press. In the rats, the electrode tips were located in the septal area, while in the cats the caudate nucleus was found to be an effective site of stimulation (*5*).

Figure 1 presents 15-minute cumulative response curves obtained from one cat under two reinforcement schedules. The curves shown are typical of those obtained during the intervening days. On the variable interval schedule the lever was connected to the stimulator at irregular intervals, with a mean of 16 sec, so that only some lever-presses produced the intracranial stimulation. On the fixed-ratio schedule, seven responses were required to produce each electric stimulus. The animals were originally trained on a continuous reinforcement schedule, in which every lever-press resulted in an electric stimulus. Marked differences in the rate of responding were obtained with the two schedules. The fixed-ratio was also characterized by typical pauses following reinforcements, although these are generally obscured in the reduced figure.

The curves of Fig. 1 are similar to those obtained with food or water reinforcement. However, the low ratios and short mean intervals at which responding could be maintained suggest comparison with small amounts of reinforcement (*6*). On the assumption that stimulus intensity may be analogous to "amount" of reinforcement, amperage was varied during an hour-long session for one cat that was producing an irregular curve on a fixed-ratio schedule of 8:1. At the start of the session the stimulus was presented at a lower amperage than was usual for this cat. Figure 2, depicting the complete record for one 60-minute session, suggests that an increase in electric stimulus intensity may act in a manner similar to an increase in the amount of reinforcement.

In addition to producing stable behavior sensitive to other variables, such as electric stimulus parameters, intermittent reward schedules also have the advantage of minimizing the influence of gross motor effects of the stimulus on the response rate. Such schedules have proved useful in studying the effects of other motivating conditions, for example,

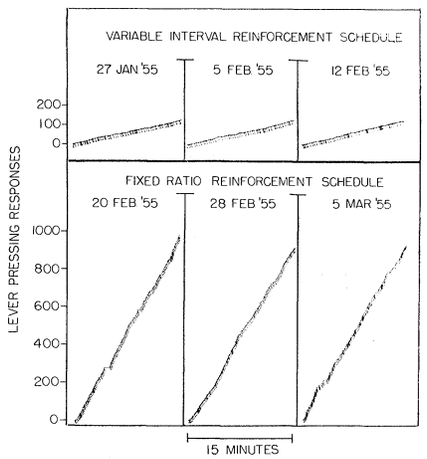

Fig. 1. Fifteen-minute cumulative lever-pressing curves for fixed ratio (7:1) and variable interval (mean of 16 sec) intracranial electric stimulation reward (cat E-5). Oblique "pips" indicate reinforcements.