ppears to be the principal origin. Theories which propose that the carbon skeletons of furfuryl alcohol and maltol of heated milk derive from carbon atoms 2 to 6 and 1 to 6, respectively, in the glucose moiety of lactose remain attractive in light of these findings (*10*).

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8 March 1957

Gamma Rays from Local Radioactive Sources

There is considerable interest at the present time concerning the possible effects of man-made radiations on man himself. Because one source of these radiations is of world-wide extent, the interest has also become world-wide. Although considerable literature now exists on the subject of man-made radioactive contamination, on the one hand, and on the biological effects of radiation, on the other, the actual importance of the first as far as the second is concerned has often been obscure. It is thought desirable at this time to present some independent experimental data that will allow individuals to reach their own conclusions.

As early as 1928, R. A. Millikan became interested in the gamma rays emitted by local radioactive materials in the soil and rock at various localities in order to determine the effect of these radiations on the cosmic-ray measurements in which he was primarily interested. These measurements extended from California into the Rocky Mountain area and on up to Churchill, Manitoba (*1*). They probably represent a unique series of measurements, since they were made before man-made contamination became widespread.

An ionization chamber measures directly the quantity of interest as far as the biological effects of gamma rays are concerned, and this is the instrument here employed. One of the instruments Millikan made and calibrated is still in good condition after 26 years and is very convenient to use. A recent redetermination of the absolute value of the calibration (*2*) agrees with Millikan's value to 0.3 percent. In this survey, Millikan's instrument has been used for some of the measurements, and a more modern ionization chamber (*3*) for others. The two give essentially the same answer. Both were used unshielded in the measurements reported here.

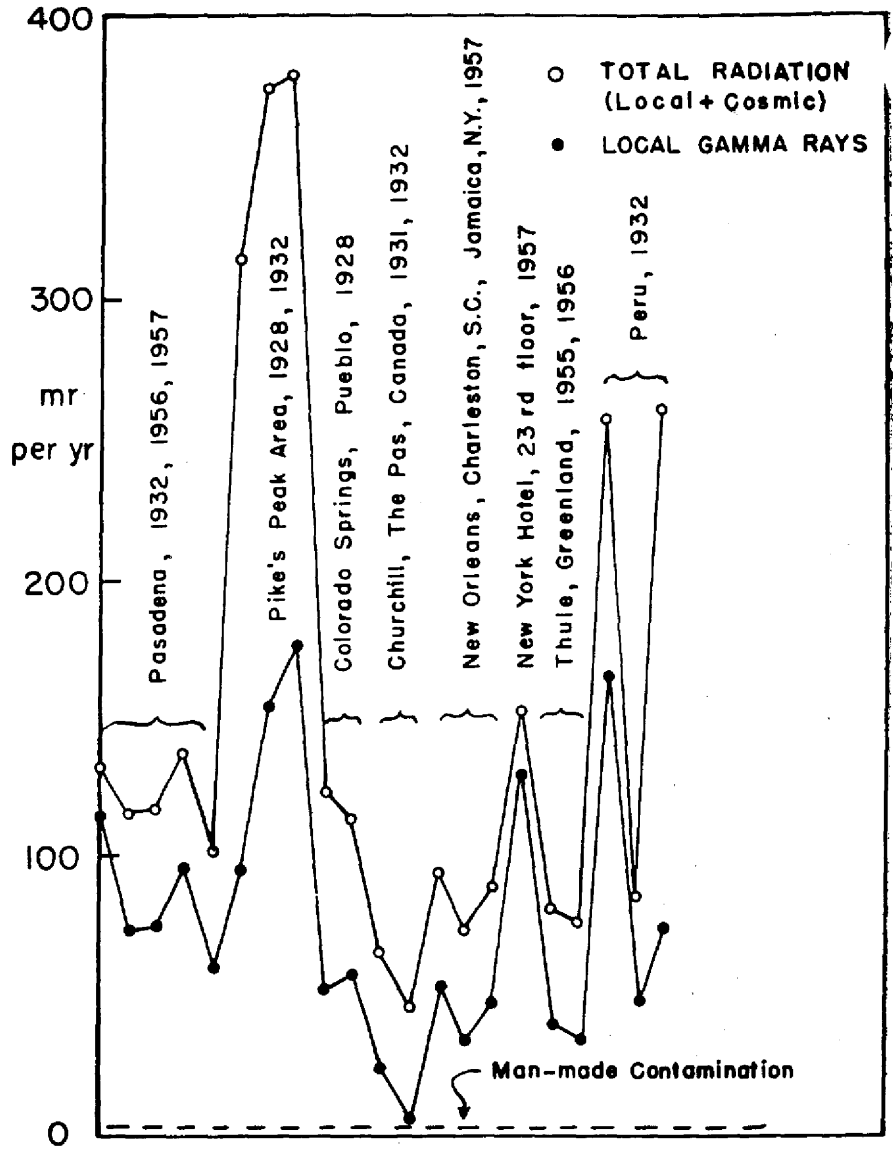

Fig. 1. "Noise level" of gamma rays and cosmic rays in the Western Hemisphere. Abscissas roughly increase with increase of distance from Pasadena. The amount of man-made contamination is taken from the National Academy of Sciences report, *Biological Effects of Atomic Radiation* (*7*). As is stated in that report, ". . . U.S. residents have, on the average, been receiving from fall-out over the past five years a dose which, if weapons testing were continued at the same rate, is estimated to produce a total 30-year dose of about 0.1 roentgen" (an average of 3 mr per year).

In Fig. 1, most of the values taken during the years have been entered. The ordinates are in milliroentgens (mr) per year. To convert into ion pairs per cubic centimeter, per second in 1 atmosphere of air, divide the ordinates by 15. The various stations are plotted as abscissas with the same increment from one to the other. Roughly, the stations get farther from Pasadena with increase in abscissa. The chief reason for plotting in this manner was to bring out the variability of radioactivity from one station and region to another.

Measurements were made of the total radiation at a given station; then the known contribution from cosmic rays (*4*) was subtracted to get the effect of the gamma rays from local radiation only.

In the Rocky Mountain region, the local radiation is high, presumably because of the granite, which is known to

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10. This investigation was supported in part by grant No. G-1165 from the National Science Foundation to J. L. Liverman.

14 February 1957

Effect of Iodoacetate and Iodoacetamide on Oxygen Uptake of Heart Mitochondria

Iodoacetate and iodoacetamide have been used as specific inhibitors of the Embden-Meyerhof pathway of glycolysis, the site of inhibition being at the triose-phosphate dehydrogenase. Early reports (*1*) indicated that iodoacetate at low concentrations inhibited anaerobic glycolysis and respiration with glucose but not the oxygen uptake induced by addition of pyruvate or lactate. More recent studies (*2*) have shown that the oxidation of pyruvate may be reasonably sensitive to iodoacetate. A study of the direct effects of iodoacetate and iodoacetamide on the aerobic oxidation of pyruvate and cycle intermediates by mitochondria would provide more information on their effects on respiration and give a basis for the judicious use of a particular concentration of these inhibitors to inhibit specifically the glycolytic pathway.

The preparation of the rat heart mitochondrial suspension and the manometric measurement of oxygen uptake were made according to the methods of Montgomery and Webb (*3*). The results are summarized in Table 1. Both inhibitors at a concentration of 1.0 m*M* produced distinct inhibition with all substrates, the strongest inhibition being observed in the oxidation of pyruvate and α-ketoglutarate, which may indicate the sensitivity of systems involving coenzyme A and lipoic acid. However, the lower concentrations also produced definite inhibitions which cannot be ignored in respiratory studies. It may be noted that iodoacetate was generally more effective than iodoacetamide. In order to produce complete inhibition of triose-phosphate dehydrogenase and glycolysis, concentrations of 0.2 to 0.5 m*M* must be used in most cases, and thus the present results indicate that a complete inhibition of glycolysis is usually accompanied with some effect on respiration (*4*).

Table 1. Effects of iodoacetate and iodoacetamide on the mitochondrial oxidation of various substrates. The reaction medium contained 121 m*M* KCl, 20 m*M* potassium phosphate buffer (*p*H 6.8), 0.01 m*M* cytochrome *c*, 5 m*M* $MgCl_2$, 1 m*M* adenosine monophosphate, 0.5 m*M* adenosine triphosphate, and 5 m*M* substrate. The temperature was 37°C. The mitochondrial suspension was incubated for 10 minutes with the inhibitors in the medium, and the oxygen uptake was determined over a period of 1 hour.

Substrate	Change (%) at various concentrations		
	0.01 m*M*	0.10 m*M*	1.0 m*M*
Iodoacetate			
α-Ketoglutarate	− 6.3	−33.3	−75.6
Malate	− 7.4	−20.0	−63.9
Pyruvate + malate	− 4.3	−43.0	−85.6
Succinate	− 3.8	− 8.0	−61.2
Citrate	+ 4.6	− 8.1	−34.6
Isocitrate	+15.0	−15.3	−35.0
Iodoacetamide			
α-Ketoglutarate	− 6.4	−17.2	−76.3
Malate	− 1.0	−21.0	−35.3
Pyruvate + malate	− 9.4	−12.6	−79.7
Succinate	− 2.5	−17.0	−43.1
Citrate	−16.1	−14.9	−44.1
Isocitrate	−12.0	− 7.3	−29.1

The preparation of the rat heart mitochondrial suspension and the manometric measurement of oxygen uptake were made according to the methods of Montgomery and Webb (*3*). The results are summarized in Table 1. Both inhibitors at a concentration of 1.0 m*M* produced distinct inhibition with all substrates, the strongest inhibition being observed in the oxidation of pyruvate and α-ketoglutarate, which may indicate the sensitivity of systems involving coenzyme A and lipoic acid. However, the lower concentrations also produced definite inhibitions which cannot be ignored in respiratory studies. It may be noted that iodoacetate was generally more effective than iodoacetamide. In order to produce complete inhibition of triose-phosphate dehydrogenase and glycolysis, concentrations of 0.2 to 0.5 m*M* must be used in most cases, and thus the present results indicate that a complete inhibition of glycolysis is usually accompanied with some effect on respiration (*4*).

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18 February 1957

Carbon-14 Activity of Some Heat-Degradation Products of Milk Containing Lactose-1-C^{14}

The course of heat-induced lactose-protein interaction in milk has been followed with the aid of lactose-1-C^{14} (*1*). Use of labeled lactose also appeared attractive for investigation of the sugar's decomposition under these conditions. Of the many fragments known to be formed (*2*), formic acid, furfuryl alcohol, and maltol (3-hydroxy-2-methyl-pyrone-4) were evaluated in these experiments. It has been proposed that formic acid is derived from carbon atom No. 1 and furfuryl alcohol from carbon atoms 2 through 6 in the glucose moiety of lactose (*3*). Maltol results rather uniquely from the heat-induced interaction of reducing disaccharides with amino compounds (*2*). It has been detected in evaporated milk, baked cereals, bread crust, and roasted malt, among other places (*4*).

Table 1. Levels of C^{14} activity found in some heat-degradation products of skim milk containing lactose-1-C^{14}.

Compound	Carbon (atom/mole)	Activity of $BaCO_3$ (Count/min mg)		Lactose/product C^{14} ratio
		Found	Theory*	
Lactose	12	8.7		
Formic acid	1	53	104	1/0.51
Maltol	6	14	17.4	1/0.81
Furfuryl alcohol	5	0.8	20.9	1/0.04
Naphthyl urethane	16	0.0	6.5	
3,5-Dinitrobenzoate	12	0.0	8.7	

* Based on molar transfer of 1 atom of C^{14}.

The three compounds in question were recovered and purified from heated (121°C for 4 hours) condensed skim milk (30 percent total solids) to which lactose-1-C^{14} (National Bureau of Standards) had been added. Steam distillation was used to isolate the compounds from the heated milk. Maltol and furfuryl alcohol were recovered from this distillate by ethyl ether extraction and were purified as described elsewhere (*3, 5*). Formic acid was recovered by neutralizing a portion of the distillate to *p*H 7.5 and evaporating the solution to dryness under vacuum (*6*). The crude formate was selectively converted to CO_2 by the method of Osburn *et al.* (*7*). This CO_2, samples of furfuryl alcohol and its derivatives, maltol and lactose, the latter from the unheated product, were converted to $BaCO_3$ (*8*). Radioactivity in these preparations was determined with a windowless flow gas Geiger-Müller counter and decade scaling unit.

The data thus secured (Table 1) reveal that carbon atom No. 1 of lactose is involved in the formic acid and maltol, but not in the furfuryl alcohol. A preliminary experiment yielded essentially the same findings with the exception that some activity was detected in the furfuryl alcohol (*9*). Further investigation of the alcohol and two carefully authenticated derivatives of it, as shown in Table 1, revealed that it had no activity.

Under the rigorous heating conditions employed in these experiments, a number of carbon sources could contribute to formate; however, carbon 1 of lactose

contain something like 4 g of uranium and 15 g of thorium per ton (*5*). In Peru, the radioactivity of the coastal plain is much the same as that of the Mississippi region near New Orleans. The local radiation at an elevation of 15,000 feet in southern Peru is only slightly higher than that of the soils of the coastal plain. Most of the houses of Arequipa are built of a light rock called "tuva" which is of volcanic origin. This rock is 3 or 4 times as radioactive as the soil near Lima.

There is considerable variability of local radiation in some cases over small distances. According to Millikan (*6*), the gamma rays on the Laurentian Shield near Churchill, Manitoba, give 0.8 ion cm^{-3} sec^{-1} atm^{-1} of air, or 12 mr yr^{-1}, while nearby the intensity on the glacial sand is 35 mr yr^{-1}. It may be of interest that the radioactivity on the ice cap near Thule, Greenland, in August 1956 was less than 2 percent of cosmic rays.

A wooden building forms some shielding from local gamma rays. In my own house, the gamma rays on the first floor give 60 mr yr^{-1}, while in the back yard the intensity is 95 mr yr^{-1}. The rather high value of 130 mr yr^{-1} on the 23rd floor of a major hotel in New York is presumably owing to the material from which the building is constructed.

The root mean square "noise" level of the total radiation given in Fig. 1 is about 160 mr yr^{-1}. To find the effect on the population, the local radiation must be weighted according to the population. This has not been done. Perhaps it is fortunate that most of the population of the country lives where the radiations due to cosmic rays and local radiations are relatively low.

The dashed line near the bottom of Fig. 1 is taken from the Summary Reports on the Biological Effects of Atomic Radiation of the National Academy of Sciences (*7*). Even though there is some error in the determination of this value, as well as considerable variation of fallout over the country, it is quite evident that man-made contamination is still small compared with the changes in radiation from one part of the country to another.

The data presented here are for gamma rays only, since the walls of the ionization chamber are too thick for beta rays to penetrate, either from naturally occurring or artificially produced radioactive materials.

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15 March 1957

New Method for Detection of Human Poliomyelitis Antibodies

We have reported that if the lower edge of a strip of filter paper is placed in a suspension of a virus, the virus rises on the paper and becomes distributed in a regular, reproducible manner (*1*). The experiments described in this report show clearly that the upward spread of virus is decreased when serum containing specific antibody is placed in a band across the filter paper (*2*). Serum without antibody does not exhibit this effect. The "blocking" action of specific antibody has been observed with polioviruses and with six other viruses. The "blocking" of polioviruses by human serums which contain neutralizing antibody is type specific.

Whatman filter paper No. 3 is cut into strips 12 by 1.75 cm. Each strip is marked off by light pencil lines into 1-cm spaces (numbered 1 to 12), suspended from a rubber stopper, and autoclaved. Poliovirus cultivated in monkey kidney tissue is diluted to a concentration of 100 TCD_{50} per milliliter in 0.85-percent NaCl containing 10 percent bouillon broth. Thirty milliliters of the diluted virus is placed in a sterile bottle surrounded by ice. The serum to be tested (previously inactivated at 56°C) is then distributed evenly over spaces 3 and 4 of the filter paper. The paper is placed in the bottle containing the virus with only the lower half of space 1 below the surface of the virus suspension (see diagram of apparatus, Fig. 1). After 1 hour the strips of paper are removed, and each paper space is cut off and placed in a monkey kidney tissue-culture tube. Tissue culture tubes are incubated and observed for virus cytopathogenic effects in the usual manner. Neutralizing antibody titers of the serums used in the paper tests are determined by standard tissue culture methods.

Fifty-two successive tests (104 paper strips) with 14 human serums have given virtually identical results. Virus was detected by tissue culture on every wet space of every paper strip on which serum containing no antibody had been placed. In contrast, no virus was found above space 6 on any of the paper strips that were treated with serum which contained type-specific poliovirus antibody. No virus was detected above space 4 in the vast majority of such strips. Figure 1 shows examples of typical results. In

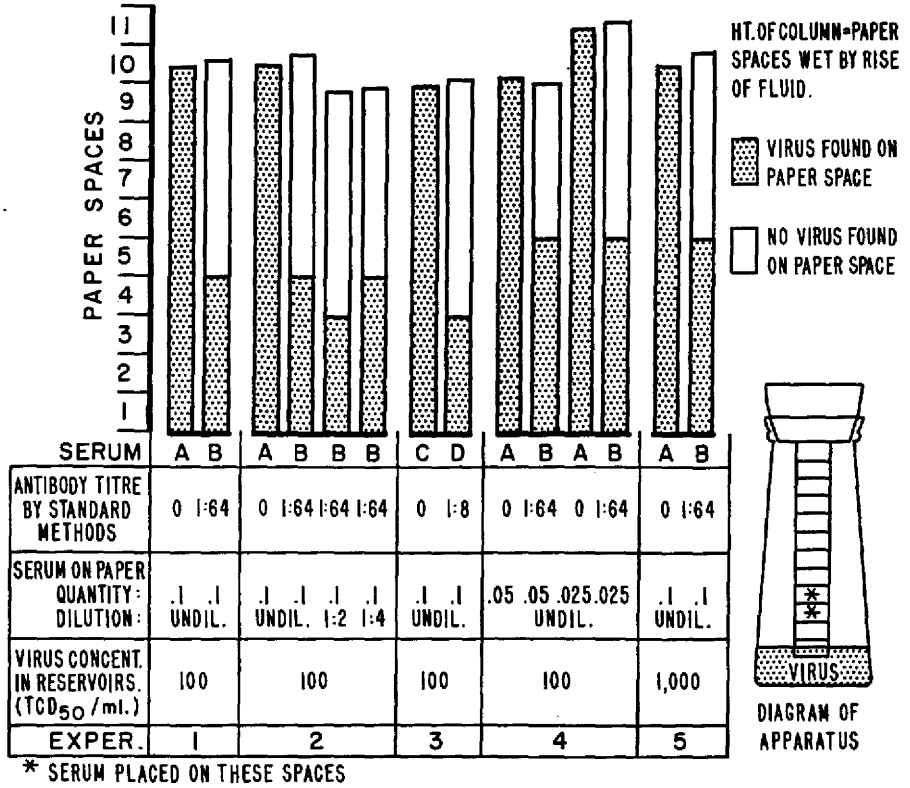

Fig. 1. Sample experiments showing that serum which contains type-specific antibody decreases the extent of the spread of type 2 poliomyelitis virus on filter paper.

experiment 1, serum A contains no neutralizing poliovirus antibody. When placed on filter paper, it did not prevent the rise of virus; all of the ten spaces which became wet were shown to contain virus when placed in tissue culture. In contrast, no virus could be detected above space 4 on the paper that had been treated with serum B, which had a neutralizing antibody titer of 1/64. Experiment 2 shows that the "blocking" action is still present when serum B is diluted. Experiment 4 shows that the "blocking" action was demonstrable with as little as 0.025 ml of serum B, a quantity readily obtainable by finger puncture.

For practical purposes, the placing of a single paper space into a single tissue-culture tube gave correct information regarding the presence or absence of poliovirus antibody. This is true of space 7, for example, in each of the 52 successive tests performed. When a pool of types 1, 2, and 3 polioviruses is tested against a serum, only that type against which there is no specific antibody in the serum can be detected high on the paper.

This method requires only one tissue-culture tube and a quantity of blood which is small enough to be obtained readily by finger puncture. The method may be, therefore, a valuable screening test for distinguishing immune from nonimmune persons in a poliomyelitis vaccination program.

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26 February 1957

Concurrent Schedules of Reinforcement in the Chimpanzee

This report (*1*) describes a technique for establishing two behavioral repertoires simultaneously in a single animal subject. This was done by training chimpanzees that had been reduced to about 80 percent of their normal weight to press either or both of two keys that were mounted 6 inches apart. The animals pressed the keys because occasional presses operated a food magazine that delivered 40-kcal. portions of food (reinforcement). The schedule by which the key presses are reinforced determines the rate at which the animal presses the key. Different rates of pressing were established on the two keys by using two schedules of operation of the food magazine (schedule of reinforcement). The schedule of reinforcement on the right key was designed to generate a high, sustained rate of pressing, whereas the schedule on the left key was designed to generate a low rate. The amount of independence between the performances on the two keys could be assessed because of the contrasting rates of key pressing. Any "confusion" between the two keys would result in high rates of pressing on the key normally producing low rates, and vice versa.

The chimpanzee, with its semierect posture and good hand dexterity, was of special interest for this type of experiment because it could operate the two keys simultaneously. Most subprimates would have to alternate between the two keys. The time spent changing back and forth between the keys would interfere with the characteristic performance under the single schedule of reinforcement.

The specific experimental conditions were similar to those already described for the pigeon (*2*). The experiment began with only one key and a schedule in which the magazine operated after a fixed number of responses. This is called a fixed-ratio schedule: "ratio" refers to the ratio of presses to reinforcements (*3*). This schedule generates a high, sustained rate of responding except when the number of responses required for reinforcement is large. Then, a pause develops following each reinforcement; but when the animal again starts pressing the key, it begins immediately at the prevailing high rate. In general, moderate rates or smooth transitions from one rate to another are absent under this schedule. If the animal operates the key at all, it tends to do so at the prevailing high rate.

After a stable performance had developed on the first key, a second key was added 6 inches to the left. Presses of the second key were reinforced on the basis of elapsed time rather than number of presses. The first press after a given interval operated the magazine; but the interval varied from reinforcement to reinforcement, ranging from 3 seconds to 8 minutes, with a mean value of 4 minutes. This schedule, which is called variable-interval reinforcement, produces a moderate rate of responding (*3*). The random spacing of the reinforcements produces a constant rate of responding and prevents pauses from developing after reinforcements. Changes in rate, when they do occur, seldom are abrupt, as they are in the fixed-ratio schedule. The variable-interval performance stabilized quickly. The number of responses required for reinforcement on the right (fixed-ratio) key was then increased to 120 over 27 experimental sessions. The larger number of responses required for

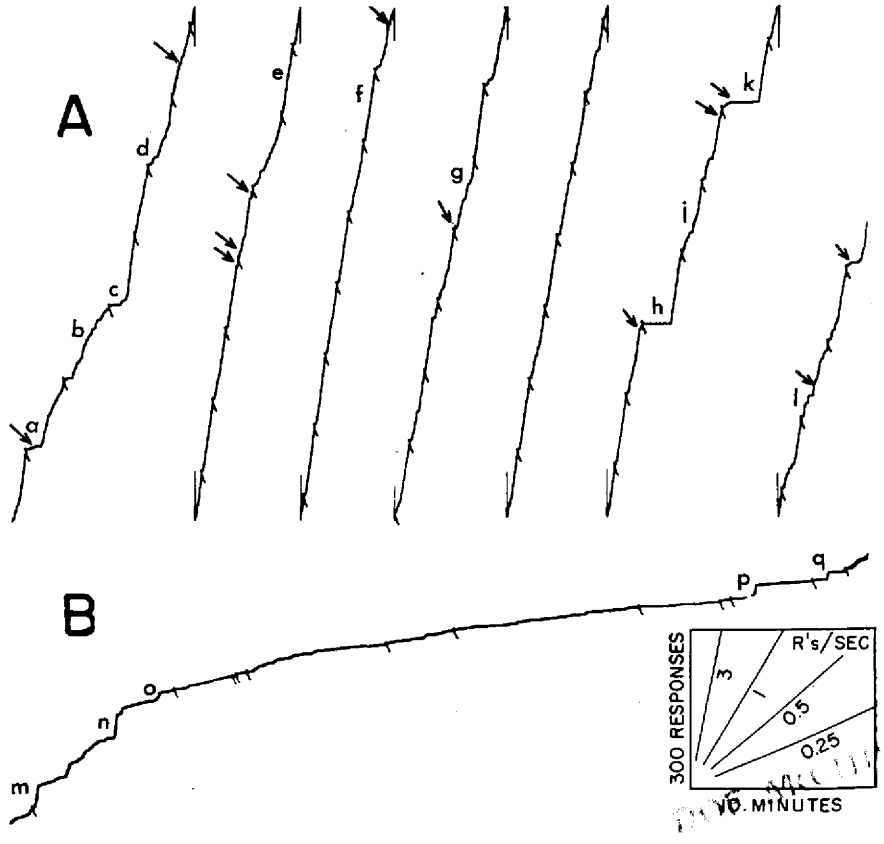

Fig. 1. Cumulative curves of responses on the two keys. Record *A*, responses on the right-hand key; reinforcement was on a fixed-ratio schedule. Record *B*, responses on the left-hand key; reinforcement was on a variable-interval schedule.