northern Illinois have been determined at 35,000 ± 2,500 and 38,000 ± 3,000 years (samples W-1450 and I-847). Because the stratigraphic position in which the artifact occurred was just above zone I in the Roxana loess and below the bulk of zones II to IV, its inferred age is in the range of 35,000 to 40,000 years.

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References and Notes

1. Ages are given in radiocarbon years before 1950.
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Immunodiffusion: Detection of a Murine Leukemia Virus (Rauscher)

Abstract. *Homologous and heterologous antiserums from several species of animals have been prepared against the Rauscher murine leukemia virus. The Ouchterlony technique, adapted to very small quantities, has been used to demonstrate at least two or three antigens in Rauscher virus preparations. Both infected-host materials and tissue-culture fluids were used as antigens. When monkey antiserum was used, one of the Rauscher virus antigens cross-reacted with an antigen in the virus strains isolated by Friend and Moloney, but there was apparently no reaction with the Moloney virus when guinea-pig antiserum was used.*

Several techniques have been applied to the problem of detection of murine leukemia viruses. When the Rauscher virus (*1*) was used as a model, methods based on infectivity (*2*), electron microscopy (*3*), and fluorescent microscopy (*4*) yielded some useful results. Infectivity assays, however, require a great deal of time, electron microscopy requires special apparatus and skills not always available, and fluorescent microscopy identifies viruses present within cells. The need for a test for rapid identification of virus in extracts of tissues and cultures may be met by the highly specific immunological technique of double diffusion, as described in this report. Several different antigenic preparations and several different heterologous antiserums as well as a homologous antiserum were used. The technique offers potential not only as a method for virus detection, but also as the most refined method available for the further delineation of the antigens of the various strains of murine leukemia virus.

Antiserums against the Rauscher virus were prepared in adult rhesus monkeys, Hartley guinea pigs, Race III rabbits (*5*), and in Balb/c mice. In general, the course for immunization consisted of a primary intraperitoneal inoculation of ten-times-concentrated plasma, obtained from Balb/c mice infected with the Rauscher virus and emulsified with an equal quantity of complete Freund's adjuvant. This was followed 21 to 28 days later by the subcutaneous inoculation of a small —0.10 to 0.25 ml—inoculum of the virus extract without adjuvant. Serum was obtained 7 to 10 days after the booster inoculation. The homologous antiserum was prepared similarly except that Formalin-inactivated virus in cell-free extracts of spleen from infected Balb/c mice was used (*6*). Control serums included those of Balb/c mice inoculated with adjuvant and saline, with extract of normal Balb/c spleen and adjuvant, or normal Balb/c serum. In all of the heterologous antiserums the following steps were used to remove antibody that might be reactive with normal plasma or with antigens, other than the virus, which were contained in the inoculum. After inactivation at 56°C for 30 minutes, Forssman antibody was removed by absorption with thrice-washed sheep erythrocytes until no reaction was demonstrable by hemagglutination. Serums were then absorbed with erythrocytes from normal Balb/c mice until no reaction was demonstrable by hemagglutination. This was followed by absorption with normal Balb/c plasma until an excess of Balb/c plasma could be demonstrated by double diffusion and no reaction occurred with the normal Balb/c plasma.

Antigens tested included the following preparations of Rauscher virus: ten-times-concentrated plasma from Balb/c mice infected with Rauscher virus (RP-10×), and a similar preparation from normal Balb/c mice as a control (NP-10×); one-hundred-times-concentrated preparations of the tissue-culture fluids obtained from a line of spleen and thymus cells grown from a Balb/c embryo, a part of the cells having been inoculated in vitro with the Rauscher virus (JLS-V5) and a part, as a control, not inoculated with Rauscher virus (JLS-V6) (*7*). Fresh 50-percent plasma from Rauscher-infected mice showing palpable spleens and fresh extracts from the palpable spleens were also tested. Other than Rauscher virus preparations, we tested one-hundred-times-concentrated plasma from Balb/c mice infected with Moloney virus (MP-100×) and a ten-times-concentrated plasma preparation from Balb/c mice infected with Friend virus (FP-10×). Moloney virus from cells infected in vivo and propagated in vitro, as described by Manaker *et al.* (*8*) and called MT-77, was concentrated 1000-fold and used as an antigen.

The Wadsworth-Crowle modified Ouchterlony technique was used (*9*). After the addition of the reactants to the gel composed of 0.85 percent ionagar, the preparations were kept at room temperature in a humidified chamber and were examined after intervals of 48 and 72 hours. Neat removal of the plastic template was greatly facilitated by chilling the preparations at 0° to 4°C for 30 minutes prior to its removal. Soaking the gel on the slide for 10 minutes with the barbital buffer recommended by Crowle made the precipitin lines more prominent. All tests were conducted in duplicate or triplicate. A permanent photographic record was made of each slide.

The RP-10× was tested with the heterologous and the Balb/c antiserums. All of these serums had neutralizing activity for Rauscher virus, as measured by the 120-day assay technique (*6*).

The monkey antiserum revealed at least two, and probably three, antigens in the RP-10×, one line of which cross-reacted with a similar preparation from Friend virus, and a hundred-times-concentrated preparation of Moloney virus. No reaction was obtained with concentrated normal plasma (Fig. 1*A*). This monkey antiserum revealed more than one band in both the

Fig.	*9 o'clock*	*12 o'clock*	*3 o'clock*	*6 o'clock*	*Center well*
A	RP-10×	FP-10×	MP-100×	NP-10×	Monkey < R
B	NP-10×	RP-10×	JLS-V5	JLS-V6	Monkey < R
C	RP-10×	JLS-V5	FP-10×	NP	Monkey < R
D	NP	Fresh RP	Fresh R spleen	Saline	Monkey < R
E	NP-10×	RP-10×	JLS-V5	JLS-V6	Guinea pig < R
F	Rabbit < R	Balb/c < R	Guinea pig < R	Monkey < R	RP-10×
G	N Balb/c serum	Balb/c < adjuvant	Balb/c < N Spleen/c < adjuvant	Balb/c < R	RP-10×

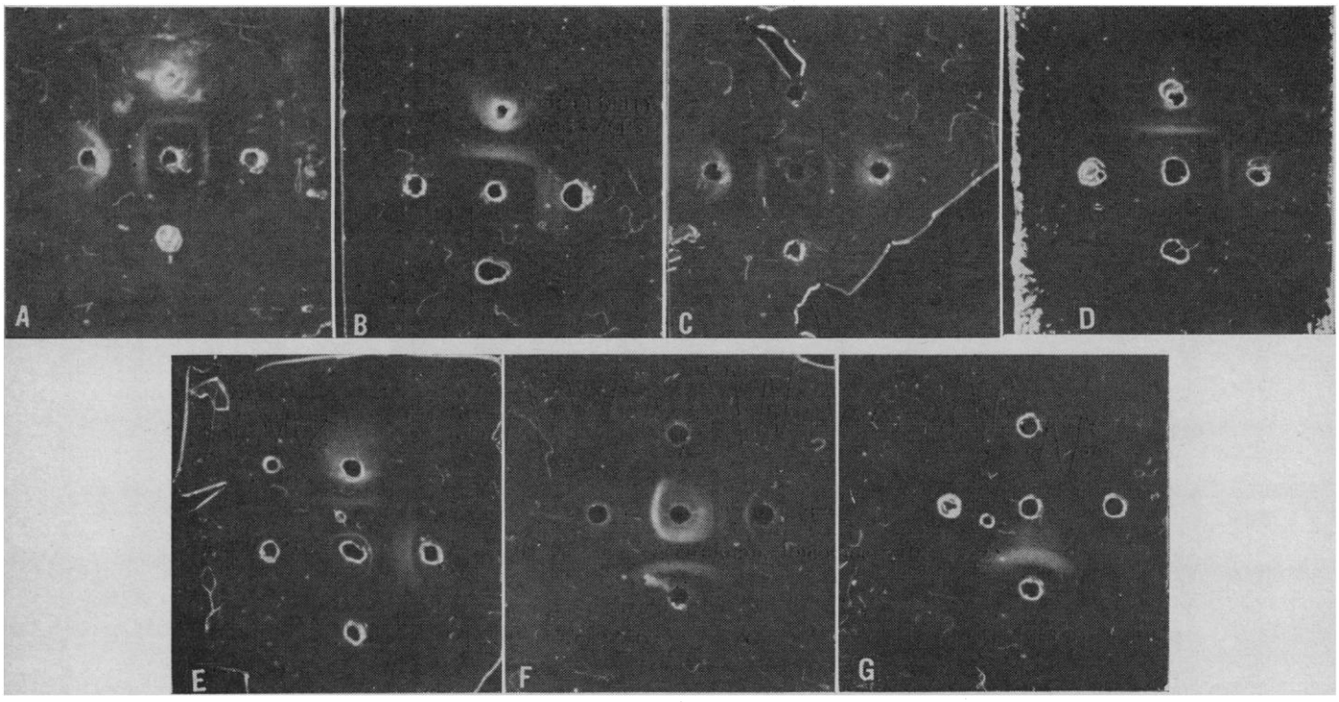

Fig. 1. Results of double diffusion tests with murine leukemia viruses. Each well contains the serum or antigen indicated in the chart above. The symbol < means antiserum to. Other abbreviations: R, Rauscher virus; F, Friend virus; M, Moloney virus; N, normal; P, plasma; JLS-V5, R tissue culture; JLS-V6, control tissue culture; 10 ×, ten times concentrated; 100 ×, one hundred times concentrated.

RP-10× and the JLS-V5, one of which definitely coincided. No reaction was obtained with control normal plasma or JLS-V6 (Fig. 1*B*). One precipitin line in Friend virus was also coincident with JLS-V5 (Fig. 1*C*).

The monkey antiserum also revealed two distinct viral antigens in the fresh 50-percent plasma of a mouse infected 84 days earlier; the virus obtained from the enlarged spleen of this mouse reacted, that is, showed lines of identity, with both of these two antigens (Fig. 1*D*). Plasmas obtained from three other mice infected 28 days previously also reacted with this antiserum, while plasma from a normal mouse did not.

With the guinea-pig antiserum there was no difficulty in distinguishing three precipitin lines in either the RP-10× preparation or in the JLS-V5, with a strong indication of a fourth band in the RP-10× (Fig. 1*E*). A cross-reaction occurred with the FP-10×; but in six attempts, we were unable to demonstrate a reaction to MP-100×.

The Balb/c antiserum apparently reacted with only one antigen of the plasma virus. The virus, however, did not react with normal Balb/c serum or the serum of Balb/c mice inoculated with adjuvant plus either saline or normal spleen extract (Fig. 1*G*) (*10*).

When the four serums were allowed to react with the RP-10×, the one line obtained by use of the rabbit serum was coincident with one of at least four lines observed with the guinea-pig serum and one of the two or three lines obtained with the monkey serum (Fig. 1*F*). Although the line with the Balb/c serum is equivocal in this photograph, one definitely occurred in a preparation incubated for 4 days.

Ten-times-concentrated plasma from mice infected with Moloney virus, in contrast to the hundred times used, gave negative results. Also giving negative results were ten-times-concentrated plasmas from mice infected with the Breyere-Moloney, Upton, and C-60 (Maraker) strains of murine leukemia virus (*11*).

We interpret these findings to mean that the Rauscher murine leukemia virus possesses more than one antigen—probably two or three—not all of which are necessarily revealed when antise-

rum prepared in only one species is used. With the homologous antiserum only one line was revealed; this line was absent when the virus was tested with normal Balb/c serum, or homologous antiserums to normal spleen plus adjuvant or to adjuvant alone. Using the more potent monkey and guinea-pig antiserums, we detected more than one line of precipitation in virus from either the plasma or tissue culture. The monkey antiserum was capable of revealing at least two similar antigens in virus from fresh, unconcentrated plasma or spleen extract of infected mice.

A cross reaction with one antigen of the Rauscher virus was observed when Friend and Moloney leukemia viruses were tested with monkey antiserum to Rauscher virus. It does not necessarily follow that these antigens are identical, since tests with antiserums to Friend and Moloney viruses have not been made; but there is a suggestion of similarity of one Rauscher antigen to an antigen occurring in the other strains. It is odd, however, that, in six attempts, a cross-reaction between the Moloney and Rauscher antigens could not be demonstrated with guinea-pig antiserum which had a neutralizing antibody titer against Rauscher virus as high as that in the monkey, and which was capable of revealing as many or more antigens in the Rauscher plasma virus. Old (*12*) has postulated that the leukemic cells derived from mice infected with Friend, Moloney, or Rauscher viruses contain a common antigen. We have discussed certain possible antigenic relationships among these murine leukemia viruses, and our inability to show a cross reaction between the Moloney and Rauscher viruses except by immunofluorescence (*13*). We have interpreted these reactions as indicating antigen of viral rather than of cellular origin (*13*). Our findings reported herein, that ten-times-concentrated plasma preparations from leukemic mice infected with Moloney, Breyere-Moloney, Upton, or C-60 (Manaker) virus were negative—each of which preparations would be expected to contain an amount of cellular debris comparable to that present in any other similar leukemic plasma—strengthens the evidence that the reaction being depicted is truly one against a viral antigen, rather than against a nonviral antigen, of the leukemic cell.

The microscale Ouchterlony test, as described, is useful for virus detection and holds potential for elucidating further the antigenic structure of the murine leukemia viruses.

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10. Very recent work in this laboratory indicates that the Balb/c antiserum contains antibody reactive with two or three antigens in plasma from mice infected with Rauscher virus.
11. We thank Drs. T. Breyere, R. Tyndall, and R. A. Manaker for these preparations.
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Acidosis: Effect on Lipolytic Activity of Norepinephrine in Isolated Fat Cells

Abstract: *Lowering of* p*H from 7.40 to 6.60 significantly decreased the rate of glycerol formation in fat cells incubated with either* l-*norepinephrine or theophylline. When these cells were incubated with both* l-*norepinephrine and theophylline, the glycerol formation proceeded at maximal rate and was quite similar at* p*H 7.40 and 6.60. These results indicate that the inhibitory effect of acidosis on the lipolytic action of* l-*norepinephrine is exerted on the process which activates lipase.*

Experiments in vivo and in vitro have indicated that the increases in free fatty acid (FFA) and glycerol concentrations, which follow epinephrine or norepinephrine administration, are significantly inhibited by acidosis (*1*). We now report on the mechanism of the inhibitory effect of acidosis on norepinephrine-induced lipolysis in vitro. Isolated fat cells were used for the study because the lipolytic activity in cell-free homogenates indicates a potential ability to release FFA (*2*) rather than the physiological level at which the lipolytic process is functioning (*3*). According to recent findings (*4*) the lipolytic process in the fat cell depends apparently upon the activity of at least three enzymes: adenyl cyclase, which forms cyclic 3′, 5′-adenylic acid (3′, 5′-AMP) (*5*) and is stimulated by catecholamines (*6*); phosphodiesterase, which inactivates cyclic 3′, 5′-AMP (*7*) and is inhibited by theophylline (*8*); and lipase, the activity of which depends upon the actual amount of cyclic 3′, 5′-AMP (*9*) (Fig. 1). The purpose of the present experiments was to determine whether it is lipase, or the process activating lipase, which is inhibited by acidosis. The rate of glycerol production was taken as an index of lipase activity.

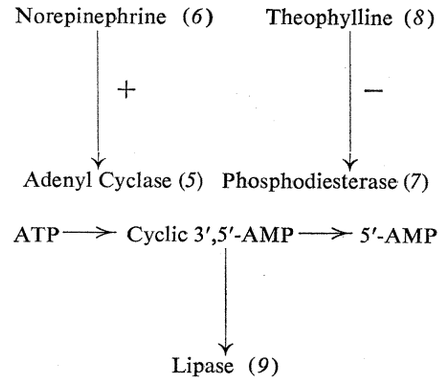

Fig. 1. Mechanism of lipase activation (this diagram does not include possible intermediate steps).

Epididymal fat pads were taken from male Sherman rats that had been permitted free access to food until they were decapitated. Isolated fat cells, prepared as described by Rodbell (*10*), were incubated at 37°C in a Krebs-Ringer phosphate buffer containing 5 percent bovine albumin, fraction V. The *p*H of this medium was adjusted either to *p*H 6.60 or to *p*H 7.40 (*11*), and the ratio of cells to medium was 1 ml of packed cells to 20 ml of medium. Suspensions of corresponding fat cells were incubated separately for each interval of time. Glycerol concentration (*12*) in the media was determined at the end of the incubation period (*13*).

In the first experiments the fat cells