Table 1. Sparse labeling of cutaneous nuclei. The results are expressed as the number of nuclei containing 3 to 15 silver grains per 1000 cells of each type. Ultraviolet irradiation, UV.

Cell type	Normal skin*		Skin from XP patients					
			Patient A		Patient B		Patient C	
	No UV	UV	No UV	UV	No UV	UV	No UV	UV
Basal cell	5.4	466	9	6	8	4	10	111
Malpighian cell	9	622	2	0	0	2	8	77
Granular cell	1.2	548	0	0	0	0	6	82
Fibrocyte	4.4	449	0	0	4	6	8	20

* Mean values for the tabulations in the eight normal control subjects.

21 days at 4°C, developed with Brussels Amidol Developer, and fixed with Edwal Quick Fixer; the tissues were then stained with hematoxylin and eosin.

A dense labeling pattern of generally greater than 20 silver grains was present in about 5 percent of basal cell nuclei of the epidermis and in about 1 percent of the dermal fibrocytes in the nonirradiated skin (Fig. 1a). The number of cells with this dense labeling did not change significantly by 15 minutes after irradiation. However, approximately half of the epidermal and upper dermal cell nuclei of the irradiated skin in the control subjects showed a sparse labeling pattern of 3 to 15 silver grains which represented the unscheduled type of DNA synthesis (Fig. 1b). In contrast, essentially no sparse labeling occurred after irradiation in the epidermis or upper dermis of the two patients with the de Santis-Cacchione syndrome (patients A and B) (Fig. 1c). A minimum amount of sparse labeling, ranging from 4 percent of normal in the upper dermal fibrocytes to 20 percent of normal in the basal cell layer, was noted in the XP patient with only skin changes (patient C) (Fig. 2 and Table 1).

The biochemical mechanisms involved in the stimulation of unscheduled DNA synthesis induced by ultraviolet irradiation in normal skin in vivo have not been elucidated. However, it has been demonstrated by density gradient techniques that this type of DNA synthesis, observed autoradiographically in cultured mammalian cells, represents the insertion of small amounts of [^{3}H]TdR into the injured DNA molecule, presumably as a result of the dark-repair process (*1–3*). We examined incorporation of [^{3}H]TdR in vivo in the skin of three patients with XP whose fibroblasts in tissue culture showed little or no evidence of DNA repair, as demonstrated by autoradiographic and density gradient methods (*2*). Unscheduled synthesis was not observed after irradiation in the epidermis or upper dermis in the two patients with the de Santis-Cacchione syndrome and was present in a small number of cells in the patient with only cutaneous lesions. These results are in close agreement with those of Cleaver, who studied cells from the same three patients. Thus, our findings support the concept that the defect in DNA repair noted in vitro occurs in vivo as well. Since defects in repair result in an increased mutation rate induced by ultraviolet irradiation in bacteria (*5*), a similar mutation increase in the skin of patients with XP after exposure to the sun might explain the cutaneous carcinogenic potential in this disease. However, a direct relation between a lack of DNA repair and cancer formation needs to be determined.

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Immune Virolysis: Effect of Antibody and Complement on C-Type RNA Virus

Abstract. *In the presence of envelope antibody and complement, the AKR strain of mouse leukemia virus was lysed, with the result that (i) the viral nucleic acid became susceptible to ribonuclease digestion and (ii) the internal group-specific antigen of the virus was released. The internal localization of the group-specific antigen is confirmed, the evidence being based on the failure of group-specific antibody to lyse virus in the presence of complement.*

Complement (C′) can enhance the virus-neutralizing ability of specific immune serum (*1*, *2*), although its precise role is still not known with certainty. The most general assumption is that C′ strengthens virus-antibody complexes by contributing additional large protein molecules to these aggregates (*2*, *3*). However, enveloped viruses having a lipoprotein component are exceptionally sensitive to the action of antibody and C′. Berry and Almeida (*4*) in a study of infectious bronchitis virus observed that the virus particles may actually have been lysed by an unheated heterotypic antiserum. These and similar observations with rubella and influenza virus were described later under the term virolysis (*5*); however, no evidence of loss of internal components, which is obtained in cellular lytic systems, was presented (*6*).

The oncogenic C-type RNA viruses also mature and "bud" from the cell surface and acquire a lipoprotein envelope in the process. In attempting to develop a rapid, sensitive assay for envelope antibodies and antigens we have obtained evidence that the combined action of antibody and complement releases or exposes viral RNA and also releases the group-specific internal virion antigen in soluble form.

Our studies were carried out with the AKR leukemia virus obtained from an in vitro cell line established from a virus-induced lymphosarcoma in the rat (*7*); however, the basic observations hold true for other C-type viruses as well (*8*). The rat cell line was grown in 32-ounce (about 1 liter) bottles in Eagle's basal medium supplemented with fetal bovine serum (10 percent) and containing glutamine (2 percent),

penicillin (100 unit/ml), streptomycin (100 unit/ml), and neomycin sulfate (0.02 percent).

Cultures were incubated with 50 ml of medium containing [^{3}H]uridine (20 μc/ml; 20 c/mmole) for 24 hours. The medium was then replaced with fresh medium without isotope for an additional 24 hours. Pooled supernatants were clarified by low-speed centrifugation, and then the virus was centrifuged at 35,000g for 2 hours. Virus pellets were resuspended in 0.01M tris buffer, pH 7.2, containing 5 percent sucrose and then centrifuged through sucrose gradients (15 to 60 percent, weight to volume; buffered with .01M tris, pH 7.2, and containing 0.1M NaCl). Centrifugation was performed in the Spinco SW 41 rotor at 40,000 rev/min for 90 minutes at 5°C. The bottoms of the tubes were punctured, fractions were collected from gradient tubes and assayed for radioactivity (Beckman LS 250 liquid-scintillation system), refractive index (Abbé refractometer), and reactivity with antibody to the virus group-specific antigen (*9*) in complement fixation (CF) tests. The group-specific antigen is an internal virion antigen and is only detectable when the virus is disrupted, for example, by treatment with ether. The small amount that is detected in purified preparations presumably results from some virus breakdown under the conditions of the CF test (overnight incubation at 5°C).

Virus initially purified by density-gradient centrifugation was then incubated with various antiserums with and without C′; after incubation at 37°C for 30 minutes, the mixtures were centrifuged again through sucrose gradients (15 to 60 percent), as described for the initial purification. The antiserums used (Table 1) were hyperimmune guinea pig serums prepared against intact AKR virus and the purified group-specific antigen (*10*), and serums from rats bearing the AKR lymphosarcoma. The antiserum to group-specific antigen has no neutralizing activity; its activity in CF tests with purified virions increases manyfold after they are treated with ether, whereas the guinea pig antiserum to whole virus and the rat antiserum appear to be envelope-specific and are nonreactive with the group-specific antigen in gel-diffusion assays.

Purified virus is not affected by incubation in buffer or with normal serum with or without C′ added; that is, the virus rebands at its equilibrium position of 1.156 g/cm^3 in sucrose (Fig. 1, upper left and right). Similar patterns were obtained with both normal guinea pig and rat serums and also, significantly, with the guinea pig antiserum to group-specific antigen. This contrasts strongly with the results obtained with the envelope antiserums. In the absence of C′, aggregates of virus and antibody are formed which sediment further into the gradient than virus alone (Fig. 1, lower left).

Virus is evidently intact under these conditions, for the RNA (as measured by the [^{3}H]uridine label) remains resistant to ribonuclease. The sedimentation patterns obtained were highly variable, depending upon antibody dilution; as expected, with relatively high antibody concentrations, most of the radioactivity was found in the sedimented pellet, whereas with increasing dilution soluble complexes were formed. The addition of C′ to antibody-virus mixtures had an obvious lytic effect on the virus (Fig. 1, lower right). No [^{3}H]uridine label was found, either in the pellet or in the region of the gradient associated with intact virus. The label remained at the top of the gradient and was almost entirely soluble in 5 percent trichloroacetic acid. This suggests that the viral RNA became accessible to ribonuclease, either on the surface of the virus particle or from the serum. In addition to release of viral RNA, the group-specific internal antigen of the virion was also released in soluble form by the addition of C′ to antibody-virus mixtures. This

Table 1. Reactivity of test serums in various assays.

Serum source	Assay		
	Neutralization*	Gel diffusion†	Virolysis‡
Guinea pig antiserum to AKR intact virus	+	−	+
Guinea pig antiserum to group-specific antigen	−	+	−
Rat bearing AKR-induced lymphosarcoma	+	−	+
Normal guinea pig serum	−	−	−
Normal rat serum	−	−	−

* In vitro assays measuring the reduction in number of transformed cell foci (*11*) produced by a pseudotype sarcoma virus bearing the AKR envelope (*12*). † This assay utilized ether-treated antigens and detects reactivity with the ether-stable group-specific antigen (*13*). ‡As described in text.

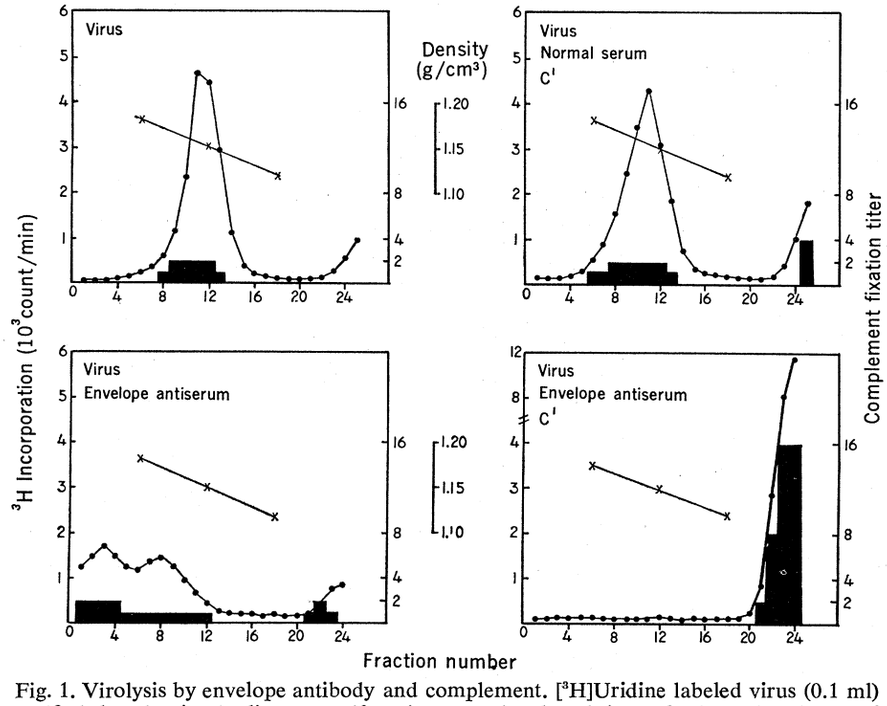

Fig. 1. Virolysis by envelope antibody and complement. [^{3}H]Uridine labeled virus (0.1 ml) purified by density-gradient centrifugation was incubated in a final total volume of 0.6 ml with tris buffer (0.01M, pH 7.4 with 0.1M NaCl) containing, as indicated, 0.05 ml of heat-inactivated antiserum (56°C, 30 minutes) or normal serum and 0.05 ml of guinea pig complement. After 30 minutes at 37°C, the entire mixture was centrifuged through sucrose gradients (15 to 60 percent, weight to volume; Spinco SW 41 rotor, 40,000 rev/min, 90 minutes, 5°C). Radioactivity is indicated by solid circles, density is indicated by crosses, and complement fixing activity with group-specific antibody is indicated by the solid bars.

antigen is of relatively low molecular weight (~25,000) and, with brief centrifugation, is found on top of the gradient; however, after overnight centrifugation through a 5- to 20-percent sucrose gradient, the group-specific antigen clearly separates from the degraded RNA.

These data support the concept of virolysis by antibody and C′ because intraviral components are released by their combined action. That antibody to an "internal" virion antigen was nonlytic, confirms the specificity of the antiserum and the true internal localization of the group-specific antigen. These observations would appear to provide the basis for a relatively rapid technique for detecting envelope antibody to C-type viruses and, by inhibition methods, the corresponding antigen. Contributions of host cell antigens to the viral envelope may also be evaluated by this method.

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Copulatory Behavior Can Inhibit Pregnancy in Female Rats

Abstract. *If female rats received genital stimulation soon enough after their male partners had ejaculated, sperm transport and subsequent pregnancy were inhibited. Manual stimulation by the experimenter or five intromissions by a male rat were sufficient stimuli to reduce the number of sperm found in the uterus and to reduce the number of uterine implantation sites.*

During copulation the male rat mounts and dismounts from the female a number of times. On some of these mounts he inserts his penis into the vaginal orifice; on the final insertion of a series he ejaculates sperm and an enzymatic coagulate, the vaginal plug, into the female's vagina. The amount of vaginal stimulation the male's intromissions provide during copulation is correlated with probability of pregnancy: the more often he intromits, the more probable it is that the female will become pregnant (*1*, *2*).

There are two major prerequisites for normal pregnancy: (i) fertilization of the eggs and (ii) secretion of hormones, such as progesterone, which permit the implantation and maintenance of the embryos in the uterus. Because this hormonal response can be initiated artificially, without the introduction of sperm, it has been called pseudopregnancy. The male's copulatory behavior promotes both pseudopregnancy and fertilization.

Fertilization depends upon the male's copulatory behavior because the intromissions before ejaculation facilitate the passage of sperm through the tightly closed cervix into the uterine lumen (*2*). The effects of such copulatory behavior on sperm transport and pseudopregnancy have been verified in other laboratories (*3*).

When taking vaginal smears, we noticed that if too much vaginal stimulation had been given to a female rat after copulation the sperm normally found in her uterus were absent. If the male rat's copulatory behavior could have such an effect, this interference with sperm transport would be a major influence on the occurrence of successful pregnancy in the rat. The experiments reported here deal with this inhibitory effect of vaginal-cervical stimulation following ejaculation, and the implications of this phenomenon for successful pregnancy.

One hundred and thirty Sprague-Dawley female rats were housed in community cages and had continuous access to food and water. To facilitate behavioral observations, we maintained the colony room on a reverse light cycle: 14 hours of lights off, 10 hours of lights on; the mid-point of the dark portion was 1:30 p.m.

To determine the number of sperm present in the uterus after copulation, we anesthetized each female and performed a laporotomy. Approximately 2 ml of Liqui-Nox was mixed with 10 ml of water; 0.1 ml of this soap solution was injected into each uterine horn, the fluid was mixed, and the entire fluid contents of the uterus were withdrawn. Sperm counts were made on a hemocytometer (*4*).

In the first experiment, 43 female rats were allowed to copulate with males until each ejaculated. The male was then removed. Normally, approximately 90 percent of such females would have become pregnant (*1*, *2*). In this experiment, however, we took the females out of the cage at various times after each had received the ejaculate, and manually stimulated the cervix with the barrel of a plastic syringe. One hour after the ejaculation, each female was killed and her uterine horns were examined for sperm.

Females were stimulated at times ranging from a few seconds up to almost an hour after ejaculation (Table 1). Cervical stimulation inhibited sperm transport most effectively if it came within 15 minutes after ejaculation. Although the median number of sperm found in the uterus 1 hour after mating was 446×10^5, this amount was reduced by a factor of four when the cervical stimulation came within 15 minutes after ejaculation; 44 percent of the females in this group had no sperm whatever in their uteri. During this initial quarter hour, inhibition was greatest during the first 4 minutes after ejaculation. (The average sperm count with stimulation during the first 4 minutes was 2.7×10^5; with stimulation between 4 and 15 minutes, the average was 272×10^5. This difference was significant at $P < .005$ by the t-test.) Sperm transport was more likely to occur if stimulation followed ejaculation by more than 4 or 5 minutes.

Since sperm counts were always made 1 hour after the male ejaculated and since the time of cervical stimulation