cells. It is therefore reasonable to suggest that the presence of the SV_{40} genome in the nuclei of the transformed cells interfered with the normal rate of synthesis of HSV.

The synthesis of HSV in proximity to the SV_{40} genome obviously suggests the possibility of hybridization such as occurs between SV_{40} and adenoviruses(10). With this idea in mind, 3 passages of HSV in transformed hamster cells (line X) were made and the virus then added to cultures of control hamster cells (line Ks). After 24 hours, when early herpes virus CPE was evident, tests for the presence of SV_{40} T antigen were made by the indirect immunofluorescence technique. Failure to detect this antigen, although far from conclusive, may suggest that hybridization of the type occurring between SV_{40} and adenovirus does not occur and is consistent with the findings of Rabson and his coworkers(11) who have lately shown that simultaneous infection of green monkey kidney cells with SV_{40} and HSV may take place, although they obtained no evidence of hybridization under these circumstances. However, they propose that phenotypic mixing with a consequent alteration of biological properties of SV_{40} might result from association under these conditions.

The demonstration that SV_{40}-transformed cells are more resistant to HSV infection taken with analogous findings in the case of adenovirus tumor cells(9) may be useful in the search for viruses etiologically associated with tumors of unknown origin. Thus if cells from a neoplasm specifically exhibited enhanced resistance to a certain virus or group of viruses, one might be encouraged to attempt to obtain additional evidence of the presence and nature of the interfering agent.

Summary. Lines of cells derived originally from various hamster and human tissues and transformed *in vitro* by SV_{40} consistently exhibited increased resistance to infection with herpes simplex virus, as compared with control cells not exposed to SV_{40}. Increased resistance did not appear to depend upon differences in cellular capacity to adsorb HSV or to allow penetration into the cell; nor could it be related to enhanced production of interferon. It is suggested that the SV_{40} genome in the nucleus of transformed cells interferes in a manner as yet undefined with the synthesis of HSV which occurs at the same site.

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The "Critical Period" for Production of Humorally Conditioned Necroses.* (31290)

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A number of qualitatively different morbid lesions can be elicited by conjoint treatment with two classes of potentially pathogenic agents: 1. Sensitizers or conditioning factors which create a specific predisposition for cer-

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tain types of tissue reactions; 2. Challengers which make this latent disease proneness manifest. Thus, in calciphylaxis, conditioning by parathyroid hormone or vitamin-D compounds permits the subsequent production of soft-tissue calcification by chemical challengers such as certain metals or mast-cell dischargers(1). In the systemic form of calcergy, intravenous injection of certain metals (particularly lead salts) induces a rather similar type of morbid predisposition in which massive calcification occurs at sites where mast-cell dischargers, serotonin or histamine are administered(2). An essentially different form of humoral conditioning is exemplified by the thrombohemorrhagic phenomenon (THP) in which pretreatment with another group of metals or with sulfated polysaccharides produces a thrombohemorrhagic diathesis; here challenge by catecholamines, octapressin, 5-HT or mast-cell dischargers precipitates the formation of thrombi usually accompanied by hemorrhages(3). Thus mast-cell dischargers (*e.g.*, dextran, cpd. 48/80, polymyxin) or mast-cell products (5-HT, histamine) can act as challengers in calciphylaxis, calcergy and the THP. Preliminary observations have shown that if these compounds are administered systemically as sensitizers, they induce a specific predisposition for the production by various agents of almost exclusively necrotic lesions(4).

Most forms of calciphylaxis—like the thrombohemorrhagic lesions of the Sanarelli-Shwartzman phenomenon—depend upon the lapse of a definite time interval, the "critical period," between sensitization and challenge; other conditional lesions (*e.g.*, systemic calcergy, most forms of the THP) are obtained only if sensitizer and challenger are administered simultaneously. The experiments to be described here were designed to determine the "critical period" for production of humorally conditioned necroses.

Materials and methods. Two hundred female Sprague-Dawley rats with a mean initial body weight of 100 g (range 90-110 g) were subdivided into 20 equal groups and treated as indicated in Table I.

For the purpose of humoral *conditioning*, we gave: 5-Hydroxytryptamine "5-HT" (Serotonin creatinine sulfate, Nutritional Biochemicals, Cleveland, Ohio) 1 mg, Compound 48/80 (Burroughs Wellcome & Co., Montreal, Canada) 2 mg and Polymyxin-B sulfate (Abbott Laboratories, Montreal, Canada) 2 mg, all administered subcutaneously on the belly in 0.2 ml of distilled water, while Dextran (M.W. 75,000, a 6% solution stabilized by 5.7% sorbitol N.F., Abbott Laboratories, North Chicago, Ill.) was injected into the jugular vein in 1 ml. As a *challenger* we invariably administered 200 mg of NaCl in 2 ml of distilled water deep into the connective tissue of the shaved back. The dextran and NaCl injections were performed under light ether anesthesia. Numerous control experiments showed that in otherwise untreated rats NaCl thus administered causes no appreciable cutaneous necrosis. In the present series the conditioners were given at varying intervals before ("—" hrs), at ("0" hrs) or after ("+" hrs) the time of treatment with NaCl (taken as "0" hrs). Twenty-four hours after the injection of NaCl, the animals were killed with chloroform and specimens of the injection sites were fixed in Susa solution saturated with picric acid for subsequent staining with the PAS and multipurpose techniques(5).

TABLE I. "Critical Period" for Production of Humorally Conditioned Necroses.

Groups	Conditioner	Time of conditioning (hr)*	Necrosis (mm)
1	5-HT	—17	0
2	"	— 1	21 ± 3.8
3	"	0	27 ± 2.4
4	"	+ 1	3 ± 1.8
5	"	+17	4 ± 1.6
6	Dextran	—17	4 ± 1.2
7	"	— 1	11 ± 2.1
8	"	0	30 ± 1.3
9	"	+ 1	2 ± 1.2
10	"	+17	7 ± 2.2
11	Cpd. 48/80	—17	2 ± 1.7
12	" "	— 1	26 ± .8
13	" "	0	32 ± 2.4
14	" "	+ 1	0
15	" "	+17	0
16	Polymyxin	—17	0
17	"	— 1	27 ± 1.7
18	"	0	31 ± 1.6
19	"	+ 1	3 ± 1.6
20	"	+17	1 ± .6

* The challenger used was always hypertonic NaCl solution given s.c., as indicated in the text. Time of challenge is taken as "0 hours."

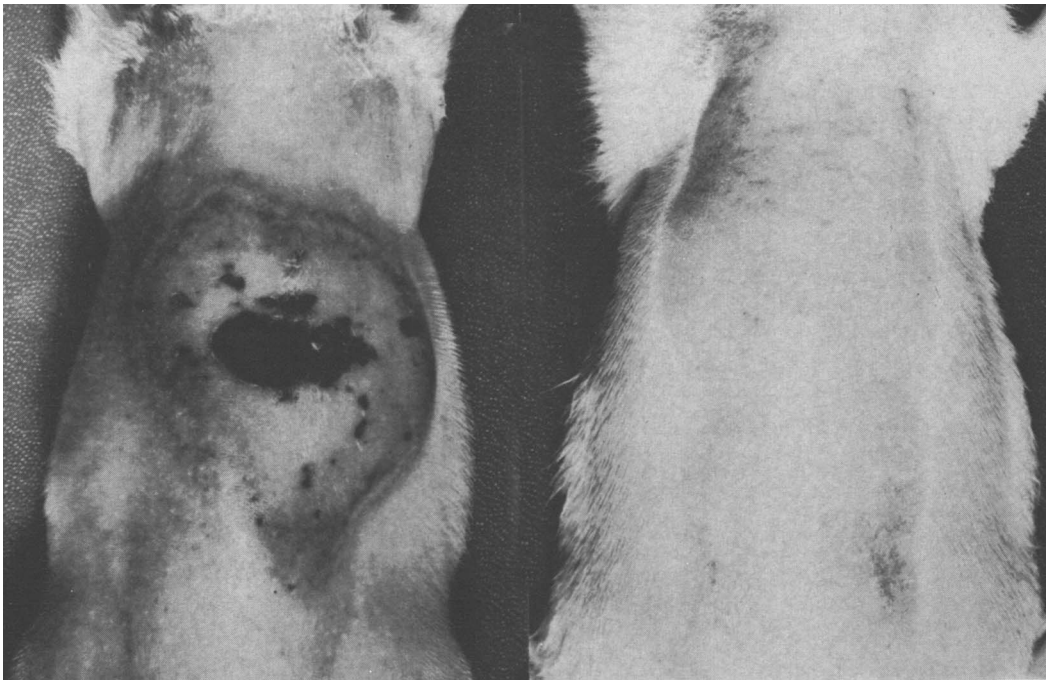

FIG. 1. Humoral conditioning for cutaneous necrosis. Both rats were treated with dextran i.v. and hypertonic NaCl solution s.c. on the back. Left: Both treatments given simultaneously: extensive cutaneous necrosis. Right: Dextran given 1 hr after NaCl: skin normal.

Results. As shown in Table I, if a hypertonic NaCl solution is administered 17 hours before or 17 hours after any one of the 4 conditioners tested, it causes little or no skin necrosis, the latter being maximal when the NaCl is injected simultaneously with the conditioner. Extensive skin necrosis is also obtained by treatment with conditioners 1 hour before but not 1 hour after injection of NaCl. Apparently in order to be effective the conditioner must be administered either simultaneously with or shortly before the hypertonic saline solution; presumably the latter is rapidly absorbed, leaving no permanent tissue damage that could be turned into necrosis by 5-HT or mast-cell dischargers.

Irrespective of the evocative treatment, the damaged tissue always exhibited essentially the same structural characteristics. Macroscopically, the lesion manifested itself in the form of a roughly circular patch showing bluish discoloration and edema within about 30 minutes after injection of the hypertonic solution. During the next 24 hours this same region disintegrated, the epithelium being lost and the underlying dermis transformed into a semitransparent parchment-like hard scab (Fig. 1). Histologically, the outstanding characteristics of this lesion were bland necrosis with thrombosis of the veins and venules, occasional small hemorrhages, edema of the subcutaneous tissue and intense discharge of the mast-cell population in the surrounding area.

Discussion. It is evident that, with the techniques used, humorally conditioned necrosis could be elicited only during a very short critical period. The same was found to be true in preliminary experiments in which these and other mast-cell dischargers were given in combination with concentrated solutions of glucose or urea. It must be remembered, however, that such hypertonic solutions produce tissue damage either immediately or not at all since they are rapidly diluted by fluid coming from the blood and by the absorption of the solutes. At near physiologic concentrations NaCl, urea and glucose are well tolerated by connective tissue; it is only their hypertonicity that exerts a potentially necrotizing action. It is quite possible,

however, that the critical period would be longer with irritants causing a more permanent tissue damage and with longer acting conditioning agents. Thus it is known that the more chronic inflammatory response to croton oil can be turned into necrosis at almost any time by heavy overdosage with glucocorticoids(6,7).

It was noted some time ago that in rats conditioned with 5-HT, an otherwise well-tolerated ligature of the abdominal aorta causes extensive necrosis in the skeletal musculature affected by the reduction of blood supply(8). Hitherto unpublished work revealed furthermore that subcutaneous injection in normally well-tolerated amounts of several tissue irritants other than hypertonic solutions (*e.g.*, ethanol, $ZnCl_2$) also causes massive skin necrosis in rats conditioned with 5-HT or mast-cell dischargers. Apparently, just as the development of inflammation is subject to regulation by pro- and anti-inflammatory hormones so the resistance of tissues to necrosis can be decisively influenced by humoral agents. Any future attempt at the further elucidation of this control mechanism will have to take the critical period into account since (especially with short-acting tissue irritants) reactivity largely depends upon the length of time elapsing between administration of the conditioner and challenger.

Summary. Normally well-tolerated doses of hypertonic NaCl produce extensive tissue necrosis in the rat if administered simultaneously with serotonin or such mast-cell dischargers as dextran, cpd. 48/80 or polymyxin. This predisposition for necrosis is demonstrable only if the conditioning agent is administered during a brief "critical period" simultaneously with or shortly before the hypertonic solution.

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Unique Specificity of Mouse Angiotensinogen to Homologous Renin. (31291)

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It is well known that renin of one species may release angiotensin I, the precursor of angiotensin II, from renin substrate (plasma) of other animals. The reaction, however, does not proceed at equal rates between species. Substrates of primate origin resist conversion by non-primate renin(1), but substrates from non-primates are thought to react readily with all mammalian renins studied(2). In the white mouse, we have found a unique resistance of mouse angiotensinogen (plasma) to conversion to pressor substances by renin from all other species tested. In contrast, mouse renin reacts promptly with heterologous substrate. In other respects (response to nephrectomy and salt-loading), the mouse renin-angiotensin system resembles those of other mammals.

Material and methods. Inbred male white mice (Charles River strain) with weights of 25-50 g were routinely fed commercial ("Nafag") pellets and tap water *ad libitum*. In sodium-loading experiments, the pellets were soaked in 0.9% NaCl (500 ml/2.5 kg of pellets), then dried, and the animals given 1.0%

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