the whereabouts of the virus during the latent period in the SV_{40}-hamster system are all questions for the future.

Summary. Intracerebral and subcutaneous injection into newborn hamsters of vacuolating virus, SV_{40}, grown in renal cell cultures of grivet monkey resulted in single or multiple fibrosarcomas at site of injection which were histologically of varying degree of malignancy. These occurred 3½ to 8 months post-inoculation and in both sexes. Animals injected with appropriate control materials or held uninoculated failed to develop tumors. Tests to exclude mouse polyoma virus as a factor were clearly negative. Evidence for the role of SV_{40} virus as a primary oncogenic agent was provided by recovery of the virus from the tumor and by demonstration of SV_{40} antigen in tumors by fluorescent antibody staining. The agent appeared to be localized in the tumors since the virus was not detected in blood or excretions, since antibody response was minimal or lacking, and since gross metastases were lacking. Transplantation and serial passage of SV_{40}-induced tumors were accomplished with ease. The data represent the first definitive evidence implicating a virus of primate origin as a malignant oncogenic agent in experimental animals. The findings relate to observations of oncogenesis in hamsters and do not warrant extrapolation to oncogenesis in other mammalian species.

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A New Model of Skin Injury.* (27299)

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The increased vascular permeability at inflamed skin sites is invariably preceded and accompanied by vasodilation(1). This observation suggests a causative relationship, even though it has been demonstrated that capillary permeability is a function of the intracapillary blood pressure rather than the capillary diameter(2).

According to one view(3), the passage of proteins across vascular walls occurs at cellular junctions due to changes in the properties of a cement substance which was thought to be present in this location. This hypothesis was later modified in that protein extravasation was attributed to changes in the geometry and dimensions of the intercellular spaces due to the swelling of the endothelial cells which accompanies the inflammatory process(4). More recently, a hypothesis was presented, according to which the transendothelial passage of macromolecules occurs by a process akin to pinocytosis(5,6). This last view dissociates vasodilation from the transfer of protein molecules across endothelial membranes, since changes in vascular

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smooth muscle are presumably not related in a causative manner to the process of endothelial vesiculation.

An attempt was made to dissociate protein extravasation from vasodilation by applying a known phlogogen to skin areas subjected to the action of a vasoconstrictor agent. In general, a known amount of epinephrine was injected intradermally and shortly thereafter xylene was applied topically. This latter agent consistently produces an intense inflammatory response in the rat within a few minutes after its application. When intravenously injected T 1824 was used to sharpen the contrast between constricted areas and the surrounding inflamed skin, it was found that both epinephrine and norepinephrine diminish the severity of the reaction in doses of 0.001 to 0.01 μg contained in a volume of 0.05 ml of physiological saline.

Early in these studies it was observed that the skin areas subjected to both epinephrine vasoconstriction and xylene inflammation differ in some respects from normal skin, as well as from skin treated with only one of these agents. It was felt that a systematic analysis of these interrelations would further our understanding of the various mechanisms involved in maintenance of vascular homeostasis.

Methods. Male albino rats of the Nelson strain were used in these studies. The reaction as obtained routinely was elicited as follows. The animals were anesthetized with sodium pentobarbital (35 mg/kg body weight) and the abdominal skin clipped. Usually 6 abdominal skin sites were then injected, each with one microgram of epinephrine in 0.05 ml of physiological saline. Following the appearance of vasoconstriction at injected sites, the left abdominal side (containing 3 vasoconstricted areas) was painted once with xylene. The animals were returned to their cages after permitting the inflamed skin to dry so as to prevent any transfer of the phlogogen to control areas. The inflammatory reaction thus induced is of short duration, lasting 2 to 4 hours. About 24 hours later, long after the xylene inflammation had subsided, the animals were again anesthetized and injected intravenously with 0.5 ml of a 1% solution of T 1824, following which xylene was applied topically to the entire abdominal skin. This test application of xylene involves the following areas: a. normal skin, b. skin treated a day earlier with xylene, c. skin treated a day earlier with epinephrine, d. skin treated a day earlier with both xylene and epinephrine. Only the skin areas belonging to the last category do not blue, indicating the absence of protein extravasation.

Results. The number of animals subjected to most procedures is small (no less than 4), but since each animal provided a number of various control sites, the results below are considered valid. The following is a summary of various observations regarding the properties of the refractory skin areas.

Areas pretreated with both epinephrine and xylene do not respond to a test inflammation with xylene for about 4 days, the tissue gradually returning to its normal condition. The sequence of administration of epinephrine and xylene matters little. When epinephrine is injected after the xylene inflammation becomes evident, the refractory areas are less sharply delineated and more diffuse than in the first case. This effect is presumably due to the greater spread of epinephrine from the site of injection in inflamed skin.

The minimal dose of epinephrine necessary to obtain this protracted refractory condition appears to be the same as was found to inhibit the xylene induced inflammation, that is 0.001 to 0.01 μg in 0.05 ml of physiological saline. Norepinephrine is as effective as epinephrine and quantitatively shows the same activity. Pitressin produces as good a result as epinephrine at dose of 0.1 unit. Marsilid, a monoamine oxidase inhibitor, is much less effective when used in place of epinephrine. The low efficacy of Marsilid may be related to the low levels of endogenous epinephrine normally present in the skin. However the possibility of involvement of endogenous epinephrine was not substantiated in tests involving animals in an alarm reaction stage of stress provoked by subjecting them to 350 revolutions in a Noble-Collip drum. Local injection of BOL-148, a serotonin antagonist was also without effect. Menadione and hydroquinone appear

to exhibit some activity, but catechol cannot substitute for epinephrine.

Reserpine which releases serotonin from mast cells(7), is without effect, as is histamine or serotonin when used instead of epinephrine. Also ineffective is angiotensin, presumably because the initial local vasoconstriction is rapdily replaced by vasodilation and increased vascular permeability at the injected site. The fact that local dilators are ineffective when used together with xylene emphasizes vasoconstriction as a prerequisite for development of refractoriness.

When epinephrine or pitressin was used as a vasoconstrictor, histamine could not be substituted for xylene in inducing skin refractoriness. Heat (water vapors at 70°C) or cold (ethyl chloride spray) inflammation was also used as a substitute for either the initial or test application of xylene. However, the results were not clear due to the severity and prolonged persistence of the ensuing inflammatory reaction.

Since the extent of the blueing reaction is a function of both increased vascular permeability and blood flow to the affected area(8), it was necessary to ascertain whether the refractoriness to xylene was not due to the persistence of ischemia in areas which had been pretreated with both xylene and a vasoconstrictor agent. To this end, the preparatory routine was modified by injecting the skin of the lower abdomen with a vasoconstrictor at closely spaced intervals. This procedure resulted in formation of a large area of vasoconstriction because of the confluence of the individual constricted skin sites. The left abdominal side was then painted with xylene. The abdominal skin thus consisted of 4 quadrants, 3 of which served as controls. In other respects the methodology was the same as described previously. The variously treated skin areas were tested a day later with intradermal injections of serotonin, histamine or 48/80, as well as with xylene. Only the first 2 agents induced blueing in the skin area pretreated with xylene and either epinephrine or vasopressin, indicating persistence of vascular reactivity to these agents as well as the presence of blood flow. The extent of the blueing reaction was lesser, however, and the reaction took longer to develop than in the control skin areas. Following 48/80 no blueing appeared. Judged by visual inspection, 48/80 actually produces vasoconstriction in the refractory test area. The results are presented in the accompanying tables.

Discussion. The mechanism responsible for the peculiar skin refractoriness reported here seems to involve both humoral and nervous factors. The production of this state depends on presence of a pronounced vasoconstriction. Vasopressin was as effective as epinephrine or nonepinephrine. Vasoconstriction probably intensifies the injurious effects of xylene by interfering with the flushing action of the circulation, as well as with the normal exchange of metabolites between the site and blood. The vasoconstriction induced by epinephrine alone differs from that

TABLE I. Effect of Treatment on Subsequent Inflammatory Reaction to Xylene.

Treatment	Extent of blueing (T 1824)	Treatment	Extent of blueing (T 1824)
None	++++	Alarm reaction	++++
Xylene	+++	*Idem* & xylene	+++
Epinephrine, 1 μg	++++	" & epinephrine, 1 μg	++++
Idem & xylene	—	Marsilid, 1 μg & xylene	++
" & histamine, .001-100 μg	+++	BOL-148, 1 μg & xylene	++++
Norepinephrine, 1 μg	++++	Hydroquinone, 1 μg & xylene	++
Idem & xylene	—	Menadione, 1 μg & xylene	++
Pitressin, .1 unit	++++	Catechol, 1 μg & xylene	++++
Idem & xylene	—	Serotonin, 1 μg & xylene	++++
" & histamine, 1 μg	++++	Histamine, 1 μg & xylene	++++
Angiotensin, .5 μg	++++	Reserpine, 1 μg & xylene	++++
Idem & xylene	++++		

TABLE II. Effect of Treatment on Subsequent Reactivity of Skin Sites.

Treatment	Extent of blueing, following application of test substance (T 1824)			
	Histamine, .1 μg	Serotonin, .1 μg	48/80, .1 μg	Xylene
None	++++	++++	++++	++++
Xylene	++++	++++	+++	+++
Epinephrine, 20 μg/ml	+	+	—	++++
Idem & xylene	+	+	constr.	—
Norepinephrine, 20 μg/ml	+	+	—	++++
Idem & xylene	+	+	constr.	—
Pitressin, 10 u/cc	++++	+++	++++	++++
Idem & xylene	++	++	constr.	—

due to vasopressin in that it subdues the subsequent reactivity of the site to the local injection of histamine and serotonin and inhibits almost entirely the blueing normally induced by 48/80. This observation indicates that epinephrine may release skin amines as reported in the literature(9) in addition to its known vasoconstrictor action. Since peripheral vasoconstriction is normally effected by adrenergic discharge, the diffuse glandular system of mast cells may be regarded as responding to an increase in vascular tone. The depletion of skin amines obtained by the combined action of a vasoconstrictor and xylene must be considerable, if not absolute, a situation which does not obtain in animals subjected to a routine depletion schedule with 48/80(10).

The vasoconstriction induced in the refractory area by 48/80 may represent the direct action of this compound on the terminal vascular bed. The structural similarity which 48/80 (low condensation products of p-methoxy-N-methylphenylethylyamine and formaldehyde) bears to epinephrine may be responsible for its vasoconstrictor action, the effect in normal skin being masked by the vasodilation elicited by the amines which are released by this compound from mast cells (11). The constrictor action of 48/80 observed in this study is in agreement with the reported antagonism this substance exhibits towards histamine and acetylcholine in the small intestine(12), and with the conclusion that the toxicity of 48/80 is apparently not related to its histamine liberating action(13).

The role played by xylene is of major importance. The inflammation produced by this agent resembles that of histamine in the sequence of movements of water and protein out of the blood vessels(14). Its action can only partly be ascribed to release of skin amines, since it still induces blueing, albeit a weaker reaction, in rats subjected to a rigorous depletion with 48/80 (unpublished observation). The injury produced by xylene is partly dependent on the presence of intact sensory innervation, involving in all probability the activation of axon reflex arcs(15). The partly neurogenic inflammatory action of xylene is consonant with the known role played by the antidromic conduction in sensory nerves in the etiology of herpes zoster, as well as the demonstration that intense nerve stimulation can elicit the appearance of hemorrhagic lesions(16).

The observation that histamine, which readily elicits the axon reflex(17) cannot substitute for xylene when injected intradermally, must be viewed in the light of possible deleterious effects of xylene upon subsequent activation of the axon reflex(15).

It is suggested that the skin refractoriness following the combined action of xylene and epinephrine is due to the depletion of skin amines and abolition of the axon reflex. This explanation accounts for the disappearance of normal skin responses to both xylene and 48/80, as well as for the concomitant persistence of sensitivity to the non-neurogenic inflammatory effect of histamine and serotonin.

Summary. Topical application of a vasoconstrictor agent and xylene modifies in the rat the usual skin responses to subsequent

applications of various phlogogens. The production of this state is believed to involve the peripheral neurohumoral system.

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Oxethazaine and Related Congeners: A Series of Highly Potent Local Anesthetics. (27300)

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N,N-bis- (N-methyl-N-phenyl-t-butylacetamido)-β-hydroxyethylamine HCl (Oxethazaine, WY-806) is a representative of a new series of highly potent local anesthetics(1). This agent together with the N-substituted congeners prepared from mephentermine* shown in Table I contain a glycine amide moiety and were developed from approximately 300 glycine amides of the mono- and bis-acetamide configuration(2) wherein the features of the procaine and lidocaine types of anesthetics were incorporated in the molecule. Characterization of such molecules show that the mono-acetamides tend to resemble procaine in activity while the bis-acetamides of the oxethazaine type exceed by far the potency of cocaine, procaine, dibucaine, lidocaine or S-650.† Moreover, oxethazaine is unique in that as a weak base it is relatively non-ionized in acid solutions so that its efficacy at pH 1 or gastric pH is high while that of other anesthetics is negligible.

Methods. Topical anesthesia was determined as described by Sollmann(3) and consisted of filling the conjunctival space with 0.4 ml of the test solutions and then holding the eyelids together for 1 minute to bathe the cornea. For *infiltration* effectiveness 0.2 ml was injected into the inner canthus(4). Groups of rabbits were used at each of several concentrations and the opposite eye similarly treated with either distilled water or physiological salt solution served as the control. Anesthesia was determined by testing for presence or absence of the wink reflex by gently stroking either the cornea (topical) or

* Wyamine,® Wyeth Labs., Inc.

† Supplied by Ayerst, McKenna & Harrison Limited (Canada).