the kallikreins are probably splitting kallidinogen at a specific peptide link of which arginine is one component.

The esterolytic activity of acetone-activated plasma kallikrein is of particular interest because of the many esterases which have already been tentatively identified in human plasma. Of those esterases known to be inhibited by SBTI, *e.g.*, plasmin, thrombin-E, thrombokinase and the permeability factor, only plasmin has been shown not to be activated by acetone(3). The observation that intravenous infusion of thrombin-E(12) did not alter the blood pressure in dogs clearly differentiates this proteinase from plasma kallikrein. In addition, although definitive data are lacking, it would appear unlikely that thrombokinase(14) and plasma kallikrein were identical. The premeability factor(13), on the other hand, cannot be definitely distinguished from plasma kallikrein. There are, therefore, at least 5 proteinases present in human plasma-plasmin, thrombin-E, thrombokinase, C'_1 component of complement and plasma kallikrein. To these can be added the acetone-activated proteinase(s) which are not inhibitable with SBTI since they differ from C'_1 in not attacking tyrosine esters. Austen (15) has also noted the presence in chloroform-activated human serum of a TAMe esterase which is not inhibited by SBTI.

Summary. Evidence is presented which suggests that the kallikreins derived from hog pancreas, human pancreas, human urine and human plasma are capable of digesting those synthetic esters which contain arginine as the specific amino acid residue. Highly purified hog pancreatic kallikrein also digests acetyl-L-tyrosine ethyl ester. Arginine esters and benzoyl-L-arginine amide prevented both inhibition of human urinary and pancreatic kallikreins by human plasma and inhibition of human plasma kallikrein by diisopropylfluorophosphate. Acetone activates not only plasma kallikrein but another proteolytic enzyme(s) which attack arginine esters and are not inhibited by SBTI.

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Tumors Induced in Hamsters by Injection of Rhesus Monkey Kidney Cell Extracts. (26576)

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Previous experiments have shown that hamsters <1 to 3 days of age are susceptible to tumor induction by the SE polyoma virus, (1) a virus isolated from the mouse. Since a large number of simian viruses have been recovered from monkey kidney tissue cultures (2-7) the present experiments were undertaken to find out if rhesus monkey kidney

cell cultures harbor a virus also capable of inducing tumors in hamsters.

Materials and methods. Cell extract inocula. Extracts refer to frozen and ground tissue culture cells that had been concentrated by centrifugation, or tumors frozen and ground and diluted with a small amount of balanced salt solution. Primary rhesus monkey kidney cell cultures prepared in the Tissue Culture Section of the Division of Biologics Standards were originally grown in medium containing 2% calf serum, then washed and nourished with synthetic medium 199(8) to which penicillin G and streptomycin sulphate were added to make 100 units and 100 μg/ml respectively and incubated at 36°C for a period of 2 weeks. The nutrient fluids were changed at the beginning of the period and again at the end of one week. At the end of the second week approximately 90 to 95% of the fluid was decanted and centrifuged. The bottles containing the remaining cell sheets were frozen and thawed until all the cells were loosened from the glass. The cell suspension and the sedimented cell debris were then transferred to a cooled mortar, frozen in dry ice and ground while frozen. After thawing, portions of one lot were filtered through a Corning fine-sintered glass filter. The material was injected subcutaneously into <1 to 3 day old hamsters in 0.2 ml doses. Each cell extract was derived from cultures made from pooled cells of 8 to 32 pairs of monkey kidneys. Similar extracts were prepared from human or feline tumors or human cell lines nourished with a defined medium containing 5% horse serum.

Hamsters. Hamsters (*Cricetus auratus*) were raised in the Animal Production Section of Nat. Inst. of Health and were kept in an area free of animals infected with the SE polyoma virus. The animals were injected when <1 to 3 days of age with 0.2 ml doses of cell extracts, cells or tissue culture fluids and cared for in the same manner as the hamsters injected with the SE polyoma virus(1).

The tissues of animals that contained tumors were fixed, either in sodium acetate buffered formalin or in sodium acetate buffered mercuric chloride formalin ("B5")(9), embedded, sectioned and stained with hemotoxylin and eosin or with Masson's trichrome technic(10) or Bielschowski's method for reticulum (Foot's modification)(11).

Transplantation experiments. Portions of hamster tumors were removed aseptically and placed in Earle's balanced salt solution (12) containing twice the amounts of penicillin G and streptomycin sulphate used in the culture medium. After 30 minutes at 20°C the tissue was minced finely with scissors and injected subcutaneously into <1 or 3-day-old hamsters.

Tissue cultures for culturing hamster tumor material. Three kinds of cell cultures were employed in an attempt to propagate a virus from the hamster tumors: 1) a continuous line of rhesus monkey kidney cells obtained from Dr. Virginia Evans and maintained with synthetic medium 109(13), 2) primary mouse embryo cell cultures(14) and 3) primary vervet monkey kidney cultures prepared in the Tissue Culture Section by Younger's trypsinization method(15).

Extracts of tumor tissue or minced tumor tissue from hamsters were added to the cell cultures and these were incubated at 36°C and examined weekly for cytopathogenic changes. The nutrient fluids were changed at weekly intervals and passages were made to new cell cultures at 2 to 7 weeks.

Hemagglutination and hemagglutination-inhibition tests. To doubling dilutions of virus or tissue extracts an equal volume of 0.5 ml of 0.4% washed guinea pig erythrocytes in 0.85% sodium chloride solution were added and the mixtures were incubated at 4°C until the cells settled in patterns on the bottom of the tubes as previously described (16). Subcutaneous tumor tissue, liver, and kidney tissue of a given hamster injected with monkey kidney cell extract were each prepared for use in the hemagglutination test by grinding a known weight of tissue with alundum, diluting to make a 20% suspension and centrifugating at 4000 rpm for 5 minutes in an angle head centrifuge to remove the larger particles of tissue. Blood for the hemagglutination-inhibition tests was collected by cardiac puncture of the hamsters and from the femoral vein of monkeys.

Results. Incidence of tumors. Two hun-

TABLE I. Tumors Induced in Hamsters Injected When <1 to 3 Days of Age with Cell Extracts of Different Origins.

Cells origin	Lot	Hamsters inj. No.	Hamsters inj. Date	Total No. of animals surviving without tumors	Days of observation of survivors†	No. of animals with tumors at: Mo 5	Mo 7	Mo 9	Mo >9
Human tumor	A	11	6/ 2/59	5	473	0	0	0	0
Idem	B	11	6/11/59	9	494	0	0	0	0
Monkey kidney	1	10	6/11/59	1	500	5	8	8	9
Idem	2	13	6/11/59	2	500	3	7	10	11
Cat on human cell lines	X	6	6/19/59	4	293	0	0	0	0
Human tumor	C	9	7/ 6/59	9	459	0	0	0	0
Monkey kidney	3	6	7/10/59	0	301	1	4	4	5
Idem	4	12	7/10/59	1	472	2	7	8	9
"	5	11	11/ 6/59	7	352	0	2	2	4
"	6	7	12/ 1/59	1	327	1	4	6	6
"	7	7	12/ 2/59	6	325	0	0	0	0
"	8	1	12/ 2/59	1	325	0	0	0	0
Human tumor	D	9	12/ 3/59	6	319	0	0	0	0
Monkey kidney	9	12	12/17/59	12	311	0	0	0	0
Idem	10	9	12/31/59	0	207	1	7	7	7
"	11	43*	1/14/60	2	283	20	40	41	41
"	12	23	1/21/60	4	349	4	7	12	17
Human tumor cell line	E	19	2/ 4/60	18	277	0	0	0	0
		219‡		88		37	86	98	109

* Fourteen of these animals were inj. with monkey kidney cell extract that had been filtered through a fine sintered glass filter.

† Maximum time of observation.

‡ Twenty-two hamsters were lost to the experiment due to early deaths.

dred and nineteen hamsters were injected with cell extracts of different origins as listed in Table I. One hundred and nine of these animals developed tumors at site of inoculation. Some hamsters were sacrificed for tissue culture or for histological examination; all others with tumors died. All the tumor-bearing animals had received extracts prepared from monkey kidney cell cultures. Of the total of 154 which received monkey kidney cell extracts 70% developed tumors. Fourteen of the hamsters had been injected with filtered monkey kidney cell extracts and 13 of these developed neoplasms which were of the same nature as those that were induced by the unfiltered cell extracts. The earliest tumor was detected 99 days after injection of the cell extract but most of the tumors developed after 7 to 9 months.

Three monkey kidney cell lots induced no tumors in 19 of 20 hamsters. One of these died from other causes before the experiment was ended. One cell lot, No. 8, was represented by only one hamster.

Transplantation of tumors. Transplants were made to newborn hamsters from 3 of the induced hamster tumors. Transplants from the first tumor injected into 12 hamsters resulted in formation of tumors in 10. These tumors appeared within 13 to 41 days and all 10 hamsters died by the 73rd day. Transplants from the second tumor gave rise to tumors in all of 28 hamsters injected between 14 and 24 days and all were dead on the 45th day. A total of 5 successive transplants were made in hamsters with cells from the second induced hamster tumor and tumors developed in all the surviving animals in from 6 to 24 days. The third induced tumor was transplanted in 12 hamsters and tumors arose in all 12 of these animals in 29 to 48 days.

Pathology. Fifteen hamsters with subcutaneous tumors were examined in detail. The growths were elevated, rounded, and usually firm. On cut surface, these masses were lobulated by invaginations of the fibrous capsule. The underlying skeletal muscle was

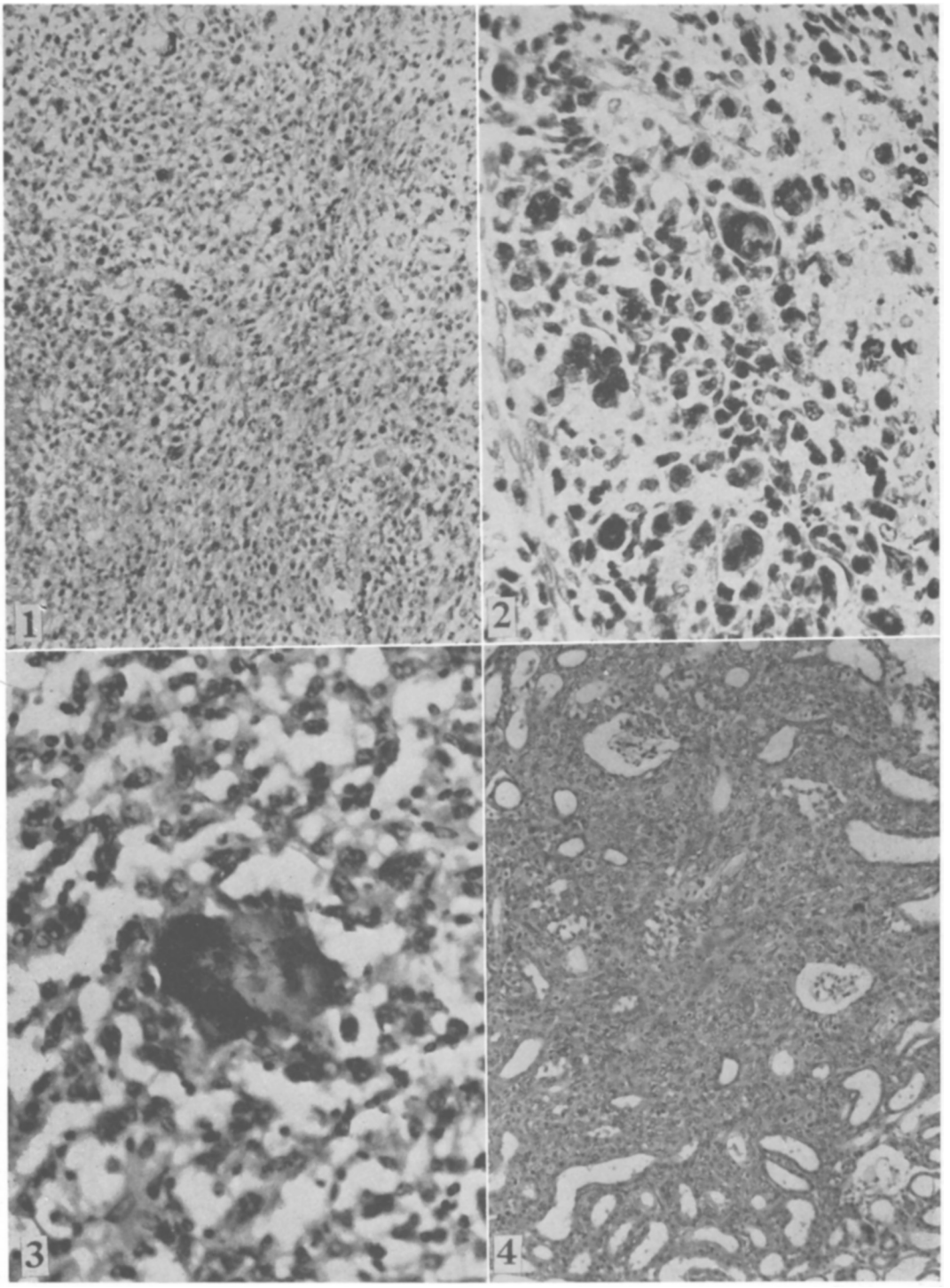

FIG. 1. Undifferentiated sarcomatous cells, some multinucleated in a section of a subcutaneous tumor in a hamster sacrificed at 223 days. H & E. Mag. 100 ×.

FIG. 2. Multinucleated giant cells within subcutaneous sarcoma. H & E Mag. 215 ×.

FIG. 3. A giant cell with basophilic nuclei in subcutaneous tissue in hamster that died at 173 days. H & E Mag. 450 ×.

FIG. 4. Rounded, vesiculated nuclei of tumor cells surrounding tubules and glomeruli in pole of kidney, in hamster sacrificed at 48 days after subcutaneous transplantation. H & E Mag. 100 ×.

TABLE II. Attempts to Culture a Virus from Tumors in Hamsters Induced with Extracts of Monkey Kidney Cells.

Origin of tumor induced by monkey kidney lot No.	Tumor No.	Tissue culture cells	No. of passages	Results
1	A 1*	Monkey kidney stable†	6	No cytopathogenic changes; no tumors developed in 2 litters of hamsters inj. with 2nd and 5th passage fluids and kept for 300 days
1	A 22*	*Idem*	4	No cytopathogenic changes
2	A 25*	"	5	*Idem*
4	A 45*	"	4	"
2	A 46*	Mouse embryo	8	"
		Monkey kidney stable	8	"
2	A 61*	*Idem*	2	"
		Mouse embryo	5	"
9	A 80	Monkey kidney stable	4	"
		Vervet monkey kidney primary	2	Cytopathogenic changes but not different from control cultures
9	A 115‡	Mouse embryo	2	No cytopathogenic changes
		Vervet monkey kidney primary	2	Cytopathogenic changes but not different from control culture
9	A 129	Mouse embryo	5	No cytopathogenic changes

* Hamsters <1 to 3 days of age inj. with the same tumor extract that was added to cell cultures failed to develop tumors when kept for 200 to 300 days.

† The monkey kidney stable cell line originated from rhesus monkey kidney cells.

‡ Cells from minced tumor A 115 were successfully transplanted to other hamsters.

occasionally infiltrated by tumor and the muscle fibers appeared to be engulfed by the tumor cells.

Three hamsters had tumors in the lungs and 2 had similar masses in the kidney.

The subcutaneous tumors consisted mainly of cells with pleomorphic, anaplastic nuclei, and variable amounts of cytoplasm (Fig. 1).

The nuclei were rounded or ovoid, usually vesiculated, with chromatin arranged as in the spokes of a wheel and/or marginated at the perimeter of the nucleus. In some tumors, there were spindle shaped cells and nuclei arranged in interlacing and whorling bundles or in a loose matrix with sparse amounts of cytoplasm. Numerous multinucleated giant cells were present in the sumors (Fig. 2). Many of these were clearly delineated with distinct rounded basophilic nuclei arranged closely within the cell. Others contained fragmented, basophilic nuclear debris within abundant eosinophilic cytoplasm (Fig. 3).

The central portions of the subcutaneous tumors frequently were necrotic. There were many congested and occasionally thrombosed blood vessels within the growths.

The tumors in the lungs and kidneys had a similar histologic pattern as described above (Fig. 4).

The neoplasms appeared to be undifferentiated sarcomas in subcutaneous tissue, kidneys and lungs. Infrequently, there were subcutaneous lobules with histological features resembling fibrosarcomas.

Virologic studies. As shown in Table II 3 kinds of tissue culture were incubated with material from the hamster tumors. No degenerative changes that could be attributed to a virus were observed and the fluids from the second and fifth tissue culture passage of one tumor did not induce tumors in 2 litters of hamsters that were inoculated and kept for 300 days.

Portions of 6 of the tissue extracts that were added to the tissue cultures were injected into litters of newborn hamsters and kept for 200 to 300 days. No tumors were

observed in any of these animals.

Tumor No. A 115, was the first tumor transplanted to other hamsters. As mentioned above, tumors developed in 10 of the 12 animals injected.

Portions of the liver, kidneys and tumor tissue of one tumor-bearing hamster were ground separately, diluted in 2-fold steps and incubated at 4°C with 0.4% washed guinea pig erythrocytes. No hemagglutination was noted.

Eighteen of the tumor-bearing animals were bled and their sera tested for hemagglutination-inhibition antibodies against 2 strains of SE polyoma virus. Inhibition did not occur.

Sera were not obtained from the monkeys at the time cell cultures were prepared but sera from 100 normal monkeys from the same colony, tested for hemagglutination-inhibition antibodies against the SE polyoma virus were negative.

Discussion. The SE polyoma virus is the only biological material, other than certain of the monkey kidney cell extracts mentioned in this report, that is capable of inducing tumors in all or almost all hamsters in a litter when injected as newborn. Histologically there are similarities between the present tumors and those induced by the polyoma virus.

The characteristics of lobulation, giant cell reactions, polygonal anaplastic cells, sarcomatous cells with a tendency to differentiate into fibrous tissue were all similar to those in tumors produced by subcutaneous injection of the SE polyoma virus in newborn guinea pigs(17) and hamsters.

The tumors of the present hamsters differed from the polyoma-virus-induced neoplasms in their distribution and cellular detail. No tumors were found in heart, stomach, intestines or liver as occur usually in hamsters inoculated with high titer polyoma virus. The larger number of bizarre giant cells and lack of frequent cellular differentiation of the tumor cells also helped to distinguish these tumors from those produced in response to the polyoma virus. The SE polyoma virus can be propagated in mouse embryo cell cultures inoculated with virus-infected hamster tissue, particularly after one or 2 passages(18) and characteristic cytopathogenic changes occur in the cells(14). Thus far, there has been no evidence that a virus multiplied in the tissue culture cells inoculated with the hamster neoplasms induced with monkey kidney extracts. If a virus in the monkey kidney cell extracts is responsible for the hamster tumors then it is not detectable in the induced hamster tumors. Newborn hamsters did not develop tumors after injection with hamster-tumor extracts. Extracts of polyoma-virus-induced tumors injected directly into other hamsters do not induce tumors(1). To demonstrate polyoma virus in hamster tumors it is necessary to increase the concentration of virus and to free it from antibody by again cultivating the virus in a suitable cell culture(18). Failure to propagate a virus in certain tissue culture lines suggests that at present the tumor-inducing material must be labeled a "substance." Extracts prepared either from human or feline tumor tissue or human tissue cultures failed to induce tumors when inoculated into newborn hamsters.

Summary. Injection of newborn hamsters with extracts of certain lots of rhesus monkey kidney cell cultures has been followed some months later by occurrence of neoplasms at site of injection in 109 of 154 hamsters. Three of the neoplasms were transplanted to other hamsters and the recipient hamsters developed tumors in 13 to 48 days. Transplants from the second tumor were passed from hamster to hamster 5 times and tumors developed in all surviving animals in 6 to 24 days. Tumors could not be induced in newborn hamsters by inoculation of extracts of the hamster tumors. No changes indicative of a virus were observed when extracts of a tumor or a tumor mince were incubated with primary mouse embryo, primary vervet monkey kidney cells, or a continuous line of rhesus monkey kidney cell cultures. The tumors appeared to be somewhat different from those induced by the SE polyoma virus.

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Significance of Plasma Glycolipid Levels in Normals and in 3 Disorders of Brain Glycolipids.* (26577)

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In 2 neurological disorders of myelin, the kidney may be involved as well as the brain. In the metachromatic form of diffuse cerebral sclerosis, sulfated glycolipids are increased in cerebral white matter, kidney tubules, and urine sediment(1 - 3). In one recent case of the globoid form of diffuse cerebral sclerosis, some kidney epithelial cells have contained unusual material, the staining of which resembles that seen in the diseased white matter(3). Sugar determinations in a heterogeneous lipid extract from one earlier case of this globoid form suggested the possibility that glycolipids might be increased in the brain(4). Recent chemical and direct experimental evidence has suggested the possibility that it may be glycolipids more in the "true" cerebroside category which increase locally in certain globoid cases(3,5). It is the cerebron-like "true" cerebrosides which cause a globoid-like response when injected into rat white matter, whereas thus far injections of other glycolipids (such as sulfatides or gangliosides) have not caused this response (3,5).

If organs as remote as brain and kidney are both involved in some type of glycolipid disorder, 2 major questions arise: (1) Does the material in the kidney merely reflect concentration by the kidney of that material from the diseased brain which has "overflowed" into the circulating plasma? (2) Or, is the material in the kidney largely independent of the plasma and simply an expression in the kidney of the same type of metabolic lesion which occurs, separately, in the brain? One important differentiating point would be the plasma glycolipid level.

The following initial study of plasma glycolipids is reported here, both because these problems of lipid deposits are becoming of wider general biological and conceptual significance and because a most unusual opportunity was presented to study simultaneously a selected series of 4 living patients with 3

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