oxytocin or vasopressin in the rat, guinea pig or rabbit.

The observations of Benson and Folley(2, 3) that oxytocin maintains involuting mammary gland of rats for a period of time in a state of active secretion following daily oxytocin administration must be explained in terms other than their suggestion that oxytocin causes discharge of lactogenic hormone from the anterior pituitary gland, attractive as it may appear.

Summary. 1) The effect of various compounds upon release of lactogenic hormone from the hypophysis in response to nursing stimuli has been studied in 14-day postpartum lactating rats. 2) Atropine, Dibenamine and Nembutal effectively blocked pituitary lactogen discharge since 30 minutes nursing evoked no decline (2.35, 2.39 and 2.46 Reece-Turner units/100 g, respectively) from previously obtained control prenursing level (2.44 R-T units/100 g). The normal level following 30 minutes nursing was previously determined as 1.66 R-T units/100 g. Since Dibenamine is a potent adrenergic blocking agent and atropine a cholinergic blocking agent, it is suggested cholinergic and adrenergic links are involved in the reflex arc responsible for lactogen discharge from rat pituitary gland. 3) Oxytocin injected i.v. into Nembutal-anaesthetized lactating rats in physiological doses failed to alter pituitary lactogen (2.49 R-T units/100 g) from the control prenursing level. Amounts of oxytocin 30 and 60 x this dose produced but a slight discharge. These results suggest oxytocin has no stimulatory effect upon pituitary lactogen release in the lactating rat, and are contrary to the recent hypothesis that oxytocin is a humoral link involved in lactogen discharge.

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Serotype Composition of the Adenovirus Group. (23776)

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A previous report(1) described 14 immunologically distinct viruses possessing the attributes of the adenovirus group(2). This communication describes 9 additional serotypes of human and monkey origin, as well as a subtype of type 7, designated 7a.

Materials and methods. Criteria for including a new virus in the adenovirus group have been described previously(1,3). All types reported here demonstrated characteristic cytopathogenicity for HeLa cell and monkey kidney tissue cultures, ether resistance, lack of pathogenicity for 1-day-old and adult mice and adult rabbits, and ability to demonstrate complement fixing antibody responses in paired serums of at least 4 humans infected with other adenovirus types. The strains designated as prototypes represent, of necessity, the first strain of the type received and tested in this laboratory. Neutralization tests were performed in tube cultures of trypsin-dispersed rhesus monkey kidney cells,* maintained in medium 199. Adenovirus neutralization procedure 2(3) was employed throughout, using the same criteria for neutralization with

* Received from Microbiological Associates, Bethesda, Md.

TABLE I. Adenovirus Types of Human Origin.

Type	Prototype strain	Isolated by	Source of prototype strain: Specimen	Source of prototype strain: Type of case
		Previously reported types(1)		
1	Ad. 71	NIH(3)	Adenoid	Hypertrophied tonsils & adenoids
2	" 6	"	"	*Idem*
3	G.B.	"	Nasal washing	Common cold volunteer
4	RI-67	Hilleman & Werner(4)	Throat "	Primary atypical pneumonia
5	Ad. 75	NIH(3)	Adenoid	Hypertrophied tonsils & adenoids
6	Ton. 99	"	Tonsil	*Idem*
7	Gomen	Berge(5)	Throat washing	Pharyngitis
8	Trim.	Jawetz(6)	Eye swab	Epidemic keratoconjunctivitis
9	Hicks	Kibrick(7)	Stool	Arthritis, rheumatoid?, myelitis?
10	J.J.	NIH(1)	Eye swab	Conjunctivitis
11	Slobitski	Kibrick(7)	Stool	Paralytic polio (Type I polio also recovered)
18	D.C.	NIH(1)	Anal swab	Niemann-Pick disease?
		Newly classified types		
7a	S-1058	NIH(Shelokov)	Throat swab	Undifferentiated respiratory infect.
12	Huie	Kibrick(7)	Stool	? Non-paralytic polio
13	A.A.	NIH(Rosen)	Stool	Well child
14	DeWit	van der Veen(8)	Throat swab	ARD
15	Ch. 38	Murray & Chang(9)	Eye swab	Conjunctivitis (early trachoma?)
16	" 79	*Idem*	*Idem*	*Idem*
17	" 22	"	"	"

TABLE II. Adenovirus Types of Simian Origin.

Type*	Prototype strain	Isolated by	Specimen
C-1	Bertha	Sabin & NIH(1)	Chimpanzee stool
M-1	S.V.$_{11}$	Hull(10)	Cynomolgus kidney tissue culture
-2	CV2	NIH	*Idem*
-3	Abin.	" (Abinanti)	Air sample from monkey room
-4	2043	(Rightsel & McLean)	Monkey kidney tissue culture

* C = chimpanzee; M = monkey.

human and rabbit serums as were previously used in tests with HeLa cell cultures, with the exception that the definitive readings were made 24 and 48 hours, respectively, after the virus control cultures had demonstrated cytopathic changes greater than 1-plus.

Results. Tables I and II record the prototype adenovirus strains. In order to give type designations to the prototype viruses originating from species other than humans, while avoiding confusion with types of human origin, it is suggested that subgroups be established within the adenovirus group, *i.e.*, types of human, chimpanzee (C), and monkey (M) origin. This separation appears justified not only because of the source from which the strains were isolated, but also because of certain differences in adaptation to tissue cultures. Thus, all types of human origin, with the exception of type 18, the D.C. strain(1), can be carried through serial passage in HeLa cell cultures without difficulty; when inoculated into rhesus monkey kidney cultures, they rapidly produce cytopathic effects, but generally cannot be carried for more than 1 or 2 additional passages without the use of concentrated inocula. Conversely, the types recovered from rhesus or cynomolgus monkeys propagate and pass well in rhesus kidney cultures, and produce rapid cytopathic changes on primary inoculation into HeLa cells, but cannot be carried in serial passage in HeLa cells. The chimpanzee type, however, passes in both monkey kidney and HeLa cell cultures.

The neutralization test results which establish each of the prototypes listed in Tables I and II as being immunologically distinct viruses, are summarized in Table III. Each of the 24 prototype rabbit antiserums was

TABLE III. Heterologous Neutralization Encountered in Classification of New Adenovirus Serotypes.

Rabbit antiserum	Titer *vs* homologous virus (reciprocal)	Neutralization of heterologous prototypes* (titer in parentheses)
Type 1	640	None
2	"	"
3	2560	"
4	320	"
5	160	"
6	320	"
7	"	7a (1:5)
7a	640	7 (1:640); 11 (1:5); 14 (1:10)
8	>80	None
9	320	"
10	160	"
11	>320	7a (1:5); 14 (1:10)
12	320	None
13	80	"
14	320	C-1 (1:10)
15	160	4 (1:40); 9 (1:10)
16	>320	1(1:5); 4(1:40); 6(1:5); C-1 (1:10)
17	320	None
18	"	"
C-1	"	14 (1:20)
M-1	80	None
-2	1280	"
-3	320	6 (1:20); 8 (1:5)
-4	>1280	None

* All 14 previously reported prototype antiserums tested at 1:20 or lower against all 10 new prototypes, and all new prototype serums tested similarly against all 23 heterologous types.

titrated against the homologous virus, and tested at a dilution of 1:5, 1:10, or 1:20 against each of the new prototypes; also, each of the rabbit antiserums to the new prototypes was tested in a similar manner against each of the previously reported prototypes. Whenever heterologous neutralization was encountered, the test was repeated and the titer determined. For conciseness, Table III lists only the heterologous reactions obtained; all the other cross tests were negative at a serum dilution of 1:20 or lower. Comparable cross neutralization tests between the previously described prototypes have been reported previously(1) and are not recorded in the table. The most noteworthy cross reactions, other than those between 7 and 7a, which will be considered in detail below, were the reciprocal cross between type 14 and the chimpanzee type, and the low titer reciprocal cross between 7a and type 11. As observed previously, occasional low titer, non-reciprocal, cross neutralization was also observed.

The 7a virus, while closely related to type 7, appears sufficiently distinct immunologically to justify its consideration as a separate adenovirus. Table IV shows results of representative cross neutralization tests with the type 7 prototype and various 7a strains, as well as representative typings of related strains. Despite variations in sensitivity of neutralization tests and different potency of rabbit antiserums, the reciprocal tests clearly indicated that the viruses designated as 7a strains were consistently different from the prototype strain of type 7 (Gomen), and were not significantly different from one another. In a series of 16 reciprocal tests of 3 7a strains (S-1058, APC-H, and Ch. 334) against Gomen, the "R" values of Archetti and Horsfall(11)† were 0.09 to 0.35, with a median of 0.22, indicating a significant difference in antigenic composition. In contrast, comparison of the same 3 7a strains with each other gave "R" values in 4 tests of >0.50, 0.71, 0.97, and >1.0, indicating no significant difference among these strains. From the data in Table IV, it is seen that the apparent difference between Gomen and the 7a strains was not simply a result of variation between responses of different rabbits. Although the Gomen serum #1, which was pooled serum of 2 rabbits, discriminated more sharply between strains than the Gomen serum #2, both serums gave generally similar results. It is noteworthy that tests with the lower titer Gomen serum #1 gave the impression of a one-way cross reaction of Gomen with S-1058, whereas when the higher titer Gomen serum #2 was used, it was apparent that Gomen virus could stimulate formation of significant antibody to S-1058.

From Table IV, it is seen that neutralization tests with different strains of the same

† $$R = \sqrt{\frac{\text{titer of serum A } vs \text{ virus B}}{\text{titer of serum A } vs \text{ virus A}} \times \frac{\text{titer of serum B } vs \text{ virus A}}{\text{titer of serum B } vs \text{ virus B}}},$$

all titers being determined in the same test.

TABLE IV. Representative Neutralization Tests with Type 7 and 7a Strains (4 Experiments). Figures represent reciprocal of neutralizing antibody titer of rabbit antiserum.

	Virus						
	Type 7 strains		7a strains				
Rabbit antiserum	Gomen (prototype) Calif., 1954 (T.O.Berge)	Sekiya, Japan, 1956 (C.Tanaka)	S-1058, Wash., D.C., 1955 (A.Shelokov)	APC-H, Salisbury, Eng., 1955 (H.J.Pereira)	Ch-334, Saudi-Arabia, 1955 (R.S.Chang)	T-439, Sheffield, Eng., 1955 (D.A. J.Tyrrell)	GL2280, Ill., 1955 (J.T. Grayston)
G* serum #1	160		5	40			
S-1058	320		320	≥1280			
APC-H	10		20	80			
G serum #1	80				5		
S-1058			320		640		
APC-H				>80	20		
Ch-334	20		20	320	20		
G serum #1	≥640		10			<10	80
S-1058	640		640			640	5120
G serum #1	640	80	5				40
" #2	2560		160				
S-1058	640	160	640				2560

* G = Gomen.

type gave rabbit antiserum titers differing by as much as 8-fold. This difference probably resulted from the fact that the tests were not performed with a predetermined dose of virus based on endpoint titrations, but used a virus dilution selected to produce a 2-day incubation period. This conclusion is supported by the data in Table V, which records the results of an experiment in which type 2 rabbit antiserum was titrated against various dilutions of a type 2 virus suspension. With decreasing quantity of virus, the apparent serum titer increased more than 16-fold; it is striking that virus dilutions having the same incubation period, 2 days, gave antibody titers differing by 8-fold. The results of this experiment are roughly comparable to those obtained by Ginsberg(12) for neutralization of type 2 adenovirus in HeLa cell cultures, using entirely different criteria for evaluating neutralization.

TABLE V. Effect of Virus Dosage on Sensitivity of Neutralization Test. Adenovirus type 2 tested against type 2 rabbit antiserum.

Virus dilution	Incubation period to CPE >1+ (days)	Rabbit antiserum titer (reciprocal)
3: 1	1	<<20
1: 1	1	<20
1: 3	2	20
1: 10	2	80
1: 30	2	160
1:100	3	320

As a consequence of this variation in sensitivity of neutralization tests, differentiation of 7 and 7a strains can be made only by comparing the titers of the 7 and 7a prototype antiserums when titrated against the virus in the same test (Table IV). However, for determining if a virus belongs to the 7-7a category, a test with a potent 7a antiserum alone would suffice. Preliminary evidence suggests that the antigenic differences detected with rabbit antiserums are reflected to some extent in human antibody responses.

In view of the reciprocal low titer cross neutralization between type 14 and the chimpanzee strain (Table III), paired serums‡ of 3 persons infected with type 14 virus, all of which demonstrated neutralizing antibody rises to type 14, were tested in the neutralization test against the Bertha strain; one person demonstrated a rise from less than 1:4 to 1:16, and the other two responded from less than 1:4 to partial neutralization at 1:4. In view of the frequency of heterologous neutralizing antibody responses in persons infected with type 14(8), these results cannot be interpreted as indicating a closer relationship of type 14 to Bertha than to other adenovirus types, but do confirm the relationship of

‡ Received through the courtesy of Dr. J. van der Veen.

adenoviruses of human and non-human origin. Further evidence of this relationship is the low level neutralization of types 6 and 8 by rabbit antiserum to the Abin. strain (Table III). Hull *et al.*(10) have recovered a number of viruses having the attributes of adenoviruses from monkey kidney tissue cultures; these agents, comprising Hull's cytopathogenic Group I, were tested against the prototype antisera of the monkey and chimpanzee types. S.V.$_{15}$ typed as M-4, and S.V.$_{23}$ as M-2. S.V.$_{11}$, S.V.$_{17}$, S.V.$_{20}$, S.V.$_{25}$, and S.V.$_{27}$ were not neutralized by any of the 5 serums. The OM86 strain, recovered from monkey stool by Cheever(13), typed as M-3.

Discussion. The multiplicity of adenovirus types, particularly the existence of closely related but distinct strains, such as 7 and 7a, raises the question of antigenic stability of the adenoviruses. Reciprocal tests of strains within types 1, 2, 4, and 6, widely separated in time and/or place, have shown no evidence of antigenic differences within these types. However, preliminary evidence suggests that there is antigenic variation within type 18 as well as type 7. It seems quite probable that adenovirus types differ in the rate at which serologic variants emerge.

From present knowledge, it does not appear that the distinction between type 7 and 7a is reflected in a difference in epidemiologic pattern. Both viruses have been recovered from military recruits, and one strain of type 7 and many strains of 7a have been encountered in the general population. The latter type 7 strain, from a case of epidemic keratoconjunctivitis, was isolated in Japan (personal communication), while 7a viruses have been isolated in the United States, Canada (14), England(15,16), France(17,18), and Saudi Arabia(9); the majority of 7a strains were from cases of pharyngoconjunctival fever, simple conjunctivitis, or febrile respiratory infections. The apparent tendency to specificity of human neutralizing antibody responses, if confirmed by more adequate testing, would be of importance in epidemiologic studies as well as in selection of proper strains for adenovirus vaccines. The differences in strains may be responsible for the variation in neutralizing antibody response to type 7 observed by Hilleman *et al.*(19).

Although it does not seem sufficiently important at present that strains of the type 7 group be routinely classified as to subtype, it is worthwhile that a standard nomenclature be employed when referring to typing of isolates. It is recommended that strains neutralized by a type 7 or 7a antiserum, but not further classified, be referred to as "viruses of the type 7 group," and that the terms "type 7" and "type 7a" be reserved for strains shown to be similar to the respective prototypes.

Summary. Seven additional adenovirus serotypes of human origin have been established, including a subtype of type 7, designated 7a. Also, 3 additional adenovirus types of simian origin are reported. It is suggested that the adenovirus group be divided into strains of human, chimpanzee, and monkey origin, with separate series of type numbers.

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Fluorinated Pyrimidines VI. Effects of 5-Fluorouridine and 5-Fluoro-2'-Deoxyuridine on Transplanted Tumors.*† (23777)

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Descriptions of the tumor-inhibitory properties of 5-fluorouracil and 5-fluoroorotic acid, members of the series of fluorinated pyrimidines developed in a collaborative research between the McArdle Memorial Laboratory of the University of Wisconsin and Hoffmann-La Roche Inc., have previously been reported (1-4). Earlier work at McArdle(5) had demonstrated that uracil-2-C14 was utilized for nucleic acid biosynthesis by Flexner-Jobling carcinoma to a greater extent than by most normal tissues. Accordingly, it appeared likely that an analog closely related to uracil might have a chemotherapeutic potential in the treatment of cancer. Because of the well-known alterations of biological activity produced by the substitution of fluorine for hydrogen in a number of classes of physiologically active compounds and because of the similarity in the Van der Waals radii of the fluorine and hydrogen atoms, a fluorinated uracil was desired. The fluorine atom was placed in the 5-position because the methyl group of thymine is attached to uracil at that locus, and it was anticipated that 5-fluorouracil would block thymine biosynthesis, as indeed is the case(1). As the result of the logical extension of studies of the fluorinated pyrimidine bases, the nucleosides, 5-fluorouridine and 5-fluoro-2'-deoxyuridine, were synthesized by Duschinsky *et al.*(6). This paper reports on the tumor-inhibitory properties of these compounds; some of their biochemical and metabolic effects have been given elsewhere(1,7-9).

Methods. The fluorinated pyrimidines were prepared and supplied by Dr. Robert Duschinsky and his colleagues of Hoffmann-La Roche Inc.(3,6), and were injected intraperitoneally unless otherwise indicated, starting one day after transplantation. The treatment ordinarily involved daily injections for 7 days. Sarcoma 180 and Adenocarcinoma 755 were transplanted by trocar into groups of female Swiss and BDF_1 mice, and the volumes measured at various times after transplantation. The justification for volume measurements and the methods of calculation are presented elsewhere(2). The Ehrlich ascites carcinoma and L-1210 leukemia were carried in ascites form in female Swiss and BDF_1 mice respectively, and the survival times of the mice were recorded. A 5-fluorouracil-resistant line of the Ehrlich ascites carcinoma was also used(2). The Novikoff hepatoma was carried by intraperitoneal transplantation of minced tissue in female Holtzman rats and their survival times were observed.

Results. Toxicity. Some information on the toxicity of 5-fluorouracil has been reported elsewhere(2). At the present time toxicity studies on the nucleosides of 5-fluorouracil have been limited to experiments in albino mice (strain IS 32). Both compounds are characterized by comparatively low toxicities

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