Summary. Gastrectomized rats developed iron deficiency because of decreased absorption of iron. Although these animals absorbed almost normal quantities of iron from test doses when they were iron replete, they were unable to increase absorption of iron appropriately in response to iron depletion. This suggested that iron deficiency occurred after gastrectomy because of an inability to adjust absorption in response to changes in the body requirement.

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Studies on Serological Relationships Between Strains of Adenovirus Types 3 and 7.* (31797)

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Adenovirus types 3 and 7 have many similar biological properties, but appear to be serologically unrelated when tested by neutralization or hemagglutination-inhibition (HI). Rowe *et al*(1,2) reported no cross-reactivity when types 3 and 7 were tested by neutralization with hyperimmune rabbit sera. Studies of reciprocal HI tests with rabbit antisera(3,4) substantiated the absence of serological relationship between the 2 types.

Types 3 and 7 have several biological properties in common including; similar neutralization rate kinetics with homotypic antiserum(5), quantity of infectious virus produced(5), length of latent period prior to multiplication(5), hemagglutination reaction (6), and type of cytopathic effect (CPE) elicited in monkey kidney tissue culture(7). Perhaps the single most important biological factor in which these 2 types resemble one another is the ability to cause tumors in newborn hamsters which contain indistinguishable "T" antigens(8).

With few exceptions the serological studies described above were carried out with prototype strains and antisera prepared to the same strains. In an attempt to better elucidate the serological relationship between strains of a given type and cross-reactions between types 3 and 7 we have studied 25 strains and their respective rabbit antisera by both neutralization and HI tests.

Methods and materials. Viruses. Strains were obtained from various sources. Most were obtained after relatively few passages; however, others had been carried extensively in the laboratory. Biographies of viruses used in this study are found in Table I.

Neutralization tests. Neutralization tests

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TABLE I. Biographies of Adenovirus Types 3 and 7 Strains Used in This Study.

Type	Strain	Source	Previous passage history	Passage history in our laboratory	Infectivity titer*
3	1392	E. Herrmann, Mayo Clinic	HeLa/3	HeLa/2	3.8
3	1461	*Idem*	RhMK/1, HeLa/2	HeLa/2	3.8
3	G.B.	See Reference (3)	HeLa/7, KB/3	none	4.2
3	1730	H. Cramblett, Ohio State	RhMK/1, SSK/2	HeLa/2	4.1
3	1329	E. Herrmann, Mayo Clinic	RhMK/1, HeLa/2	"	4.8
3	1511	*Idem*	RhMK/1, WI26/1, HeLa/1	"	5.1
3	1245	"	HeLa/3	"	4.3
3	1350	"	HeLa/5	"	4.5
3	1351	"	HeLa/4	"	3.9
3	1339	"	RhMK/1, HeLa/1	"	4.1
3	1625	"	RhMK/2, HeLa/1	"	5.7
3	1488	"	HeLa/4	"	4.8
3	1512	"	RhMK/2, WI26/1, HeLa/1	"	3.3
3	1366	"	RhMK/1, WI26/1, HeLa/2	"	5.1
3	J.F.	NIH	RhMK/23, AGMK/4	none	3.7
3	15520	K. Schell, Microbiological Associates, Inc.	DT/7, KB/3, HEK/2, KB/3	"	2.5
3–7	Takeuchi	NIH	?, HEK/1, KB/1	HEK/1	>6.5
7a	S-1058	See Reference (3)	KB/1, HeLa/1, KB/3, HeLa/1, KB/4	none	3.9
7	1611	E. Herrmann, Mayo Clinic	RhMK/1, HeLa/2	HeLa/1	6.2
7	1455	*Idem*	RhMK/3, HeLa/3	HeLa/1	5.7
7	1240	"	HeLa/2	HeLa/1	5.2
7	Gomen	ATCC	HeLa/10	HeLa/2	4.0
7	Pinckney	K. Schell, Microbiological Associates, Inc.	HEK/5, KB/4	HeLa/1	4.3
7	L.L.	*Idem*	RhMK/22, AGMK/16	none	2.3
7	14500	"	HEK/5	HeLa/1	5.0

* $Logs_{10}$ $TCID_{50}$/0.1 ml based on a 7-day test in HeLa tissue culture.

were carried out in HeLa tube cultures as previously described(9) or in primary human embryonic kidney (HEK) cultures as follows: Virus was diluted to contain 32-100 tissue culture infectious doses-50% ($TCID_{50}$)/0.1 ml at 5 days in HEK. Virus was mixed with equal parts of serial 4-fold dilutions of serum and incubated one hour at room temperature. Two-tenths of each serum-virus mixture were inoculated into 2 HEK tubes. Tubes were read when 32-100 $TCID_{50}$ were present in the test. Challenge virus was always titrated simultaneously. Neutralization titers are defined as the reciprocal of the highest serum dilution causing complete inhibition of virus CPE.

Results with HeLa and HEK neutralization tests were comparable. Ratios of the heterologous to homologous titers with type 3 or 7 strains were similar in both systems. As expected HEK neutralization titers were usually 2- to 4-fold higher than in HeLa due to the lower dosage of challenge virus needed to achieve comparable $TCID_{50}$. Since antigenic variation could be detected equally well in either system, neutralization tests were preferentially carried out in HeLa wherever possible for economic reasons.

Four-fold differences were not considered significant since titers sometimes varied within 4-fold of the geometric mean in replicate tests.

Hemagglutination-inhibition tests. Hemagglutination (HA) and HI tests were carried out by the standard macro procedure described by Rosen(6). HI titers are defined as the reciprocal of the highest serum dilution causing complete inhibition of HA.

Antisera production. New Zealand rabbits 3.5 to 4.5 kg were bled immediately prior to immunization and one week after 3 semiweekly intravenous (I.V.) inoculations of 1½ ml undiluted virus. A week later animals were boosted with 1½ ml I.V. and exsanguinated by cardiac puncture 7 days later. Blood was allowed to clot at room temperature, the clot separated and the serum stored overnight at 4°C. Serum was removed from the clot the next day and clarified by centrifugation at 2000 rpm for

TABLE II. Neutralization Titers of Adenovirus Type 7 Rabbit Antisera Against Various Strains of Adenovirus Types 3 and 7.

Virus	Neutralization titers of Type 7 rabbit antisera*				
	7a-S1058	7-1611	7-1455	7-1240	7-Gomen
7a-S1058	*160*	640	640	2560	40
7-1611	160	*2560*	640	2560	160
7-1455	160	640	*2560*	640	160
7-1240	640	2560	2560	*2560*	640
7-Gomen	40	160	160	640	*160*
3-1392	0†	0	0	0	0
3-1461	0	0	0	0	0
3-G.B.	0	0	0	0	0
3-1730	0	0	0	0	0
3-1320	0	0	0	0	0
3-1511	0	0	0	0	0
3-1245	0	0	0	0	0
3-1350	0	0	0	0	0
3-1351	0	0	0	0	0
3-1339	0	0	0	0	0
3-1625	0	0	0	0	0
3-1488	0	0	0	0	0
3-1512	0	0	0	0	0
3-1366	0	0	0	0	0

* Test carried out in HeLa cells, 32-100 $TCID_{50}$ at time of reading. Titer is the reciprocal of highest serum dilution causing complete inhibition of virus cytopathic effects.

† 0 is less than 5.

20 minutes in a refrigerated International PR-2 centrifuge. Sera from both bleedings were pooled, heat inactivated at 56°C for 30 minutes and stored frozen at −20°C until tested.

Results. Cross-neutralization with type 7 rabbit antisera. Rabbit antisera were prepared against type 7a, strain S1058 and type 7, strains 1611, 1455, 1240 and Gomen. Results of cross-neutralization tests between these sera and 19 strains of types 3 and 7 are summarized in Table II. Although some homologous titers were as high as 2560, none of the type 7 antisera contained detectable neutralizing antibody at 1:5 against any of the type 3 strains. All antisera had neutralization titers against the S1058, 1611, 1455 and 1240 strains of type 7 equal to or within 4-fold that against the homologous strain. Strain 1611 and 1455 antisera had homologous titers of 2560 and 16-fold lower titers against Gomen. Gomen antiserum had a homologous titer of 160 and an equivalent titer against 1611 and 1455.

Cross-neutralization with type 3 rabbit antisera. Reciprocal neutralization tests between 14 type 3 strains and their respective rabbit antisera are presented in Table III. Antiserum to 1339 had a homologous titer of 160 and a heterologous titer of 10 against 1730, 1329, 1351 and 1512. Otherwise results were not unlike those described above for type 7 sera, *i.e.,* all type 3 antisera neutralized homologous and heterologous type 3 strains to the same relative titer. Some antisera, *e.g.,* G.B., 1730 and 1512 were considerably lower titered than others. Whether this was caused by differences in strain immunogenicity or variations in their infectivity titers cannot be determined at this time. Antiserum to type 3, strain 1461, contained low levels of antibody to two type 7 strains and 1351 antiserum neutralized a single strain of type 7. No neutralizing antibody against type 7 strains was found in the other type 3 antisera.

Cross-neutralization tests between oncogenic strains of types 3 and 7, a type 3-7 hybrid and types 11 and 14. Next we examined by cross-neutralization various oncogenic strains of types 3 and 7, a type 3-7 hybrid (Takeuchi), and types 11 and 14. All except type 14 have been proven oncogenic(8,10,11). Results are presented in Table IV. No antigenic differences were found between type 3 strains. Takeuchi was neutralized by J.F. and G.B. antisera to a lesser extent than other type 3 strains and not at all by 15520 antiserum. Conversely, antiserum to Takeuchi had a 1280 homologous titer and a 20 to 80 titer against heterologous type 3 strains.

Antisera to G.B., J.F., and 15520 did not contain detectable antibody to types 7, 11 or 14. Takeuchi antiserum had a relatively high antibody titer to all type 7 strains but not 11 or 14.

Antisera to 4 different strains of type 7 had approximately the same antibody titer against homologous and heterologous strains including Takeuchi. No antibody to type 3 or 11 was detected and 2 sera had low level titers against type 14. Type 11 and 14 antisera shared antibody with type 7 strains and each other.

HI tests. It can be seen in Table V that antisera to type 7 strains S1058, 1240,

TABLE III. Neutralization Titers of Adenovirus Type 3 Rabbit Antisera Against Various Strains of Adenovirus Types 3 and 7.

	Neutralization titers of Type 3 rabbit antisera*													
Virus	3-1392	3-1461	3-G.B.	3-1730	3-1329	3-1511	3-1245	3-1350	3-1351	3-1339	3-1625	3-1488	3-1512	3-1366
7a-S1058	0†	0	0	0	0	0	0	0	0	0	0	0	0	0
7-1611	0	10	0	0	0	0	0	0	10	0	0	0	0	0
7-1455	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7-1240	0	10	0	0	0	0	0	0	0	0	0	0	0	0
7-Gomen	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-1392	*160*	160	160	40	640	640	640	640	2560	40	640	2560	160	—
3-1461	160	*160*	160	40	640	640	640	640	2560	40	640	640	160	160
3-G.B.	160	160	*40*	10	640	640	640	640	*160*	40	640	640	40	160
3-1730	160	160	160	*10*	640	160	640	160	640	10	640	640	40	160
3-1329	40	40	40	10	*160*	160	640	160	640	10	640	640	40	160
3-1511	160	160	160	40	640	*640*	640	640	2560	40	640	640	160	160
3-1245	160	160	160	10	160	640	*640*	640	640	40	640	640	40	160
3-1350	160	40	40	10	160	160	640	*640*	640	40	640	640	40	160
3-1351	40	40	40	10	160	160	160	160	*640*	10	160	640	10	40
3-1339	640	40	160	10	640	160	640	640	640	*160*	640	640	40	160
3-1625	160	40	160	40	160	640	640	640	640	40	*640*	640	40	160
3-1488	160	40	40	10	160	—	640	640	2560	40	640	*160*	40	160
3-1512	160	40	160	40	640	640	640	160	2560	10	640	640	*40*	160
3-1366	160	160	160	10	640	640	640	160	640	40	640	640	160	*160*

* Test carried out in HeLa cells, 32-100 $TCID_{50}$ at time of reading. Titer defined as the reciprocal of highest serum dilution causing complete inhibition of virus cytopathic effects.
† 0 is less than 5.

TABLE IV. Cross-Neutralization Titers Between Oncogenic Strains of Types 3 and 7, A Type 3-7 Hybrid and Types 11 and 14.

	Neutralization titers†									
	Sera									
Virus	3-G.B.	3-J.F.	3-15520	3-7 Takeuchi	7-Gomen	7-Pinckney	7-L.L.	7-14500	11-Slobitski	14-deWit
*3-G.B.	*80*	320	80	20	0‡	0	0	0	0	0
3-J.F.	320	*80*	80	20	0	0	0	0	0	0
3-15520	80	80	*80*	80	0	0	0	0	0	0
3-7 Takeuchi	20	5	0	*1280*	320	1280	80	1280	0	0
*7-Gomen	0	0	0	320	*320*	320	80	320	5	5
7-Pinckney	0	0	0	320	320	*320*	80	1280	5	0
7-L.L.	0	0	0	80	320	320	*80*	320	5	5
7-14500	0	0	0	320	320	1280	80	*1280*	5	5
*11-Slobitski	0	0	0	0	0	0	0	0	*320*	5
*14-deWit	0	0	0	0	0	5	0	5	20	*1280*

* Prototype strains.
† Test carried out in I°HEK cells, 100 to 320 $TCID_{50}$ at time of reading.
‡ 0 is less than 5.

Pinckney and Gomen had HI titers as high or higher against heterologous type 7 strains as against the immunizing strain. Antisera to 1611 and 1455 had homologous HI titers up to 8-fold higher than some of the heterologous type 7 strains. HI antibody to type 3 strains was not detected in any of the type 7 antisera.

Results of cross-HI tests with 14 strains of type 3 are summarized in Table VI. Antisera prepared against 1351, 1339, 1625, 1488 and 1392 have homologous HI titers up to 4-fold higher than some heterologous type 3 strains although most titers are within 2-fold. The remaining type 3 antisera have HI titers against heterologous type 3 strains equivalent to or within 2-fold that of the titer with the homologous strain. In addition, low level crossing occurred between type 7 strains and some type 3 antisera.

Discussion. Results of cross-neutralization tests have demonstrated the intratypic strain homogeneity within adenovirus types 3 and

7 and the relative absence of heterotypic relationship between these 2 types. Rabbit antisera prepared against 14 different type 3 isolates and a lesser number of type 7 strains neutralized only homotypic strains to any significant degree. Although homologous titers were sometimes higher than heterologous titers for strains within the same type these were usually no greater than 4-fold and were not considered significant since replicate tests with the same virus-antiserum varied by as much as 4-fold.

TABLE V. Cross-Hemagglutination-Inhibition (HI) Titers of Adenovirus Type 7 Rabbit Antisera Tested Against Various Strains of Adenovirus Types 3 and 7.

	Serum HI titers					
Virus	7a-S1058	7-1611	7-1455	7-1240	7-Pinckney	7-Gomen
7a-S1058	*200*	400	400	400	200	200
7-1611	800	*3200*	1600	3200	800	800
7-1455	800	1600	*1600*	1600	400	800
7-1240	200	400	400	*400*	100	100
7-Pinckney	400	800	400	800	*200*	200
7-Gomen	400	800	800	800	200	*200*
3-1272	0*	0	0	0	0	0
3-1220	0	0	0	0	0	0
3-1392	0	0	0	0	0	0
3-1461	0	0	0	0	0	0
3-G.B.	0	0	0	0	0	0
3-1730	0	0	0	0	0	0
3-1329	0	0	0	0	0	0
3-1511	0	0	0	0	0	0
3-1245	0	0	0	0	0	0
3-1350	0	0	0	0	0	0
3-1351	0	0	0	0	0	0
3-1339	0	0	0	0	0	0
3-1625	0	0	0	0	0	0
3-1488	0	0	0	0	0	0
3-1512	0	0	0	0	0	0
3-1366	0	0	0	0	0	0

* 0 is less than 10.

TABLE VI. Cross-Hemagglutination-Inhibition (HI) Titers of Adenovirus Type 3 Rabbit Antisera Tested Against Various Strains of Adenovirus Types 3 and 7.

	Serum HI titers													
Virus	3-1392	3-1461	3-G.B.	3-1730	3-1329	3-1511	3-1245	3-1350	3-1351	3-1339	3-1625	3-1488	3-1512	3-1366
7a-S1058	0*	0	0	0	0	0	0	0	0	0	0	0	0	0
7-1611	0	10	0	0	0	0	40	0	40	0	0	40	0	0
7-1455	0	10	0	0	0	0	10	0	10	0	0	0	0	0
7-1240	0	10	0	0	0	0	20	0	20	0	0	20	0	0
7-Pinckney	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7-Gomen	0	0	0	0	0	0	0	0	20	0	0	0	0	0
3-1392	*400*	80	400	400	3200	1600	6400	3200	12800	80	3200	6400	800	800
3-1461	200	*100*	200	—	800	800	1600	1600	3200	100	1600	1600	400	400
3-G.B.	200	100	*200*	200	800	800	1600	3200	3200	40	800	1600	200	400
3-1730	200	100	200	*200*	800	800	800	800	1600	80	800	1600	200	400
3-1329	100	80	100	400	*800*	400	1600	800	1600	40	800	800	200	200
3-1511	100	80	100	800	800	*400*	800	800	1600	40	800	800	200	200
3-1245	100	100	100	400	800	400	*800*	800	1600	80	400	400	100	100
3-1350	100	80	100	400	400	800	800	*800*	1600	80	800	800	100	200
3-1351	200	80	200	200	1600	1600	3200	1600	*6400*	80	1600	3200	400	400
3-1339	200	200	200	400	800	1600	1600	800	3200	*200*	1600	1600	200	200
3-1625	200	80	200	—	1600	1600	1600	1600	3200	40	*1600*	3200	400	400
3-1488	100	100	200	400	800	800	1600	800	3200	40	1600	*1600*	200	400
3-1512	100	80	100	—	800	800	800	800	1600	40	800	1600	*200*	400
3-1366	100	80	100	200	400	400	800	800	1600	40	800	800	100	*200*

* 0 is less than 10.

It was noteworthy that the prototype strain Gomen appeared less sensitive to neutralization by type 7 antisera than other type 7 strains. Antiserum prepared against Gomen, however, neutralized all type 7 strains equally well. The Takeuchi strain was neutralized by both type 3 and 7 antisera and Takeuchi antiserum neutralized both types. This is in agreement with published data by Matumoto *et al*(12). As had been previously demonstrated(2,3,4) types 7, 11 and 14 were serologically related although homologous titers were always much higher than heterologous.

Cross HI results generally agreed with neutralization data in pointing to the lack of intratypic differences between type 3 and 7 strains. Some sera had homologous HI titers 4- to 8-fold higher than heterologous strains of the same type and may reflect minor antigenic differences between some strains. It should be noted, however, that these minor differences were only in one direction. Cross-reactions occurred infrequently between types and usually were detected only at low levels. Some minor HI cross-reactions between type 3 sera and type 7 viruses were not detected by neutralization.

Most, if not all strains of type 3 and 7 are antigenically indistinguishable within their respective grouping by neutralization and HI tests. This is in contrast with information obtained in studies of type 12 strains where intratypic serological differences were observed(9). These latter differences appear to have some relation to oncogenicity(13). Perhaps neutralization tests utilizing single-shot antisera(9) or use of the adenovirus kinetic neutralization test (14), both of which have been demonstrated to provide a more sensitive way of determining strain differences will uncover some intratypic antigenic variation among the strains of types 3 and 7.

Summary. Rabbit antisera were prepared against 16 strains of adenovirus type 3 and 8 strains of type 7. Type 3 strains were indistinguishable by HI and neutralization tests. Strains of type 7 likewise were antigenically similar. There is essentially no serological crossing between types 3 and 7. The Takeuchi strain which apparently is a 3-7 hybrid was neutralized by both type 3 and 7 antisera and its antisera likewise neutralized all type 3 and 7 strains. Antisera prepared to types 11 and 14 contained neutralizing antibody to all strains of type 7 but not type 3.

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