that the differences in the proportions of other fatty acids of cholesterol esters of liver between the two breeds of pigeons correspond with changes found in serum cholesterol esters in patients with atherosclerosis and healthy humans.

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1. Young, F., Middleton, C. C., Fed. Proc., 1965, v24, 164.

2. Swell, L., Field, H., Jr., Treadwell, C. R., Proc. Soc. Exp. Biol. and Med., 1960, v104, 325.

3. Clarkson, T. B., Prichard, R. W., Netsky, M. G., Lofland, H. B., A.M.A. Arch. Path., 1959, v68, 143.

4. Lofland, H. B., Clarkson, T. B., Artom, C., Arch. Biochem. Biophys., 1960, v88, 105.

5. Young, F., Middleton, C. C., Lofland, H. B., Jr., Proc. Soc. Exp. Biol. and Med., 1964, v117, 613.

6. Clarkson, T. B., Prichard, R. W., Moreland, A. F., Circulation, 1961, v24, 1087.

7. Krell, K., Hashim, S. A., J. Lipid Res., 1963, v4, 407.

8. Stoffel, W., Chu, F., Ahrens, E. H., Jr., Anal. Chem., 1959, v31, 307.

9. Snedecor, G. W., Statistical Methods, Iowa College Press, Ames, 1956.

10. Lofland, H. B., Jr., Clarkson, T. B., Prichard, R. W., Netsky, M. G., Circulation, 1959, v20, 973.

11. Lofland, H. B., Jr., Clarkson, T. B., Circulation Res., 1959, v7, 234.

12. Schrade, W., Biegler, R., Bohle, R., J. Atherosclerosis Res., 1961, v1, 47.

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Immunoglobulin Responses to Experimental Human Infection with Adenovirus Types 26 and 27. (31613)

JAMES R. LEHRICH AND JULIUS A. KASEL

U. S. Department of HEW, PHS, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigations, Bethesda, Md.

Adenovirus types 26 and 27 have not been isolated in association with naturally occurring illness. A previous study has shown that experimental administration of these serotypes to human subjects caused an acute conjunctivitis and a prolonged, asymptomatic enteric infection(1). Each agent evoked the appearance of both homotypic and heterotypic antibody responses, with demonstrable resistance to illness after rechallenge with the same virus and after cross-challenge with the other adenoviral type. Moreover, the degree of restistance could be correlated with the level of circulating antibody.

A recent study of volunteers experimentally infected with adenovirus types 1 or 4 or inoculated with soluble antigens prepared from these serotypes has shown the appearance, during the first 3 weeks after inoculation, of antibodies in the 3 major immunoglobulin classes, IgM, IgA and IgG(2). IgM antibody activity was short-lived, while IgA and IgG antibodies were more durable. Production of heterotypic IgG antibody was also demonstrated.

The present investigation has been carried out in order to study the immunoglobulin response to experimental human infection with adenovirus types 26 and 27, which are not known to be associated with naturally-occurring illness. The immunoglobulin nature of the heterotypic antibody response was also studied, as were the immunoglobulin classes of the antibodies formed after rechallenge with homotypic virus and cross-challenge with heterotypic virus.

An unexpected finding was the fact that 19 S IgM hemagglutination-inhibition and neutralization antibodies could not be detected after fractionation of serum by either sucrose density gradient ultracentrifugation or sephadex G-200 gel filtration.

Materials and methods. Clinical procedures and inocula. Volunteers were adult male inmates of Federal correctional institutions selected and evaluated according to procedures previously described(1). For the present

study, serum specimens were selected which had previously been obtained from 2 volunteers shown to have no detectable pre-existing antibody to adenovirus types 26 and 27. Each of these subjects had received a primary conjunctival inoculation with adenovirus type 27, followed in 3 months by a secondary conjunctival challenge with this serotype. One month after the secondary challenge, a cross-challenge with type 26 was administered. Virus strains and dosages have been described (1).

Serologic tests. Procedures for hemagglutination-inhibition (HI) and neutralization tests (NT) have been described(1,3). In the present study, serum specimens and immunoglobulin pools were absorbed with human type "O" red blood cells, rather than with rat red blood cells, prior to determination of HI titers. Kaolin treatment was performed on serum specimens but not on immunoglobulin pools, and appropriate corrections for dilution were made.

Quantitative estimation of immunoglobulins. IgM-, IgA- and IgG-globulin concentrations were determined by a modification of the single radial diffusion precipitin method in agar(2). The minimum detectable concentration of IgM was 0.03 mg/ml ± 0.01 (S.D.); for IgA, it was 0.10 mg/ml ± 0.03 (S.D.); and for IgG, it was 0.10 mg/ml ± 0.08 (S.D.).

Sucrose density gradient ultracentrifugation. Zone density gradient ultracentrifugation was performed in a Spinco L centrifuge employing the SW 25.1 rotor and a preformed linear gradient ranging from 5% to 28% sucrose in 0.85% sodium chloride solution (phosphate-buffered, pH 7.2). Two ml of 1:3 dilution of serum was layered onto 30 cc of sucrose gradient in each 34 ml centrifuge tube. Centrifugation was carried out for 41 hours at 23,000 RPM, after which each tube was placed in the Union Carbide gradient analyzer* and a cannula was lowered to the bottom of the tube. The contents were pumped through the cannula to a 0.2-cm cell attached to a Beckman DB spectrophotometer measuring absorbence at 280 mμ, after which serial 1 ml fractions were collected. Localization of the 19S and 7S peaks was calculated according to the method of Martin and Ames (4) utilizing a purified human 6.8S IgG-globulin standard (isolated by DEAE cellulose chromatography), and confirmed by immunoassay of each fraction, from several runs, for IgM, IgA and IgG (Fig. 1). Prior to antibody testing, fractions 3-10 from each run were combined to form a "19S" pool, fractions 15-26 were combined to form a "7S" pool and each pool was reduced in volume to 1.5 ml by ultrafiltration.

Gel filtration with sephadex G-200. A method previously described in detail was used(2). After fractionation of each 5 ml serum sample, fractions were combined to form 3 pools, containing primarily IgM, IgA or IgG respectively. Each pool was then reduced in volume to 3.0 ml by ultrafiltration.

Results. Sucrose density gradient ultracentrifugation. After fractionation of each serum specimen, a "19S" and a "7S" pool were prepared and concentrated as described above. Immunoassays performed on these pools showed the "19S" pools to contain only IgM, 0.10 mg/ml ± 0.03 (S.D.). The "7S" pools contained IgG, 1.80 mg/ml ± 0.52 (S.D.), IgA, 0.75 mg/ml ± 0.23 (S.D.) and no IgM.

As shown in Table I, no antibody could be detected in the "19S" pools utilizing either the HI or the neutralization test procedures. Antibody was detected in the "7S" pools whenever antibody was present in serum prior to fractionation. Both homotypic and heterotypic antibody activity was present in the "7S" pools.

The pattern of 7S HI antibody response to adenovirus type 27 challenge and rechallenge, and to type 26 cross-challenge, was much the same as the pattern of appearance of antibody in unfractionated serum (Fig. 2). Two weeks after the initial challenge, homotypic and heterotypic antibody titers rose sharply in both unfractionated serum and "7S"-globulin pools. Heterotypic antibody titers were actually higher than homotypic titers in these men. No significant boosts in titers were demonstrable after rechallenge or cross-challenge. Antibody remained present in

*Union Carbide Corp., Oak Ridge, Tenn.

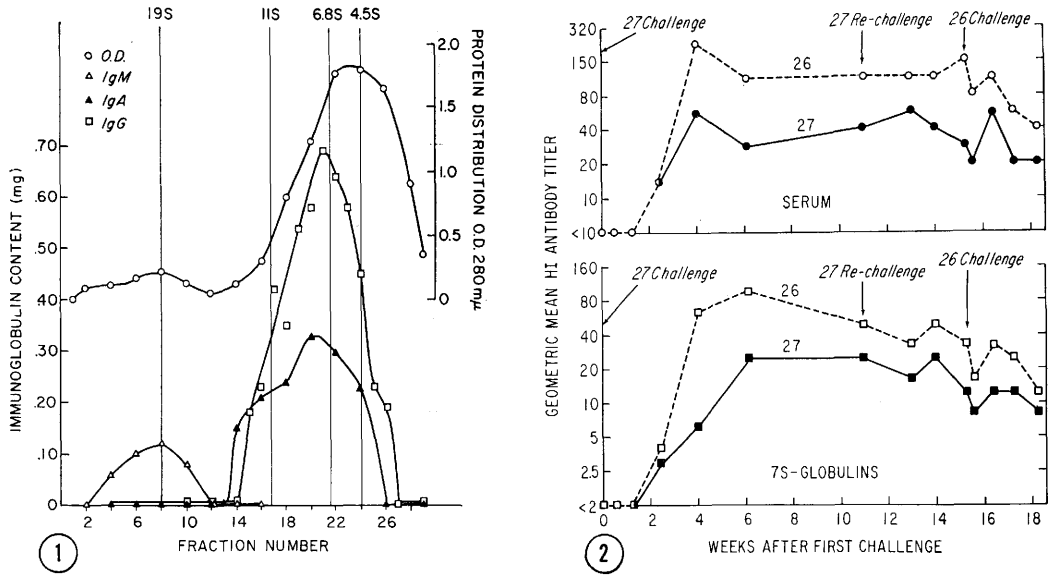

FIG. 1. Distribution of serum immunoglobulins following sucrose density gradient ultracentrifugation. Immunoglobulin concentrations were estimated by the antibody-agar plate technique. Vertical lines indicate sedimentation constants calculated according to the method of Martin and Ames(4), using purified human 6.8S IgG-globulin (isolated by DEAE cellulose chromatography) as a standard.

FIG. 2. Geometric mean hemagglutination-inhibition antibody responses in whole serum and in "7S"-globulin pools from 2 volunteers given primary and secondary challenges with adenovirus type 27 and a reciprocal cross-challenge with type 26. "7S"-globulin pools were prepared by means of sucrose density gradient ultracentrifugation, as described in text.

the "7S" pools for the 18-week duration of the study.

Gel filtration with sephadex G-200. Gel filtration techniques have been shown to produce higher yields of serum IgM-globulins than obtained by ultracentrifugation methods. Previous work from this laboratory had demonstrated IgM neutralization antibody to adenovirus types 1 and 4 using a sephadex G-200 gel filtration procedure(2). Therefore, serum specimens from one of the volunteers in the present study were fractionated by this technique and pools containing a preponderance of IgM, IgA or IgG prepared from each specimen. The "IgM" pools contained IgM, 0.48 mg/ml ± 0.13 (S.D.) and IgA, 0.42 mg/ml ± 0.04 (S.D.); the "IgA" pools contained IgA, 4.25 mg/ml ± 2.32 (S.D.) and IgG, 3.00 mg/ml ± 0.57 (S.D.); and the "IgG" pools contained IgG, 3.28 mg/ml ± 0.90 (S.D.) and IgA, 0.50 mg/ml ± 0.29 (S.D.).

As was found after sucrose density gradient ultracentrifugation, no HI or neutralization antibodies of the IgM class could be detected after gel filtration (Table II). Homotypic antibody appeared simultaneously in unfractionated serum and in the "IgA" and "IgG" pools, 2 weeks after the initial challenge. Heterotypic neutralization antibody appeared a week earlier, at which time it was found in serum and in both the "IgA" and "IgG" pools. Antibody activity then continued to be detectable in these pools for the duration of the 18-week study.

Discussion. When serum specimens from volunteers given adenovirus types 26 and 27 were fractionated by sucrose density gradient ultracentrifugation or by sephadex G-200 gel filtration, no HI or neutralization antibodies of the 19S IgM class were detected. This is in contrast to a previous report from this laboratory, a study of volunteers experimentally infected with adenovirus types 1 or 4 or inoculated with soluble antigens prepared from these serotypes(2). In that study, IgM, IgA and IgG antibodies appeared simultaneously during the first 3 weeks after inoculation. IgM

TABLE I. Immunoglobulin Distribution of Hemagglutination-Inhibition and Neutralization Antibodies After Sucrose Density Gradient Ultracentrifugation of Serum from Volunteers Challenged with Adenovirus Types 27 and 26.*

Weeks after first challenge →		Antibodies† to Adenovirus Type 27										
		0	1	2	4	11	13	14	15	16	17	18
Unfractionated serum	HI‡	0	0	15	60	40	60	40	30	60	20	20
	NT§	0	0	++	++	++	++	++	++	++	++	++
"19S"‖	HI	0	0	0	0	0	0	0	0	0	0	0
	NT	0	0	0	0	0	0	0	0	0	0	0
"7S"‖	HI	0	0	3	6	24	16	24	12	12	12	8
	NT	0	0	0	++	++	++	++	++	++	+	++

Weeks after first challenge →		Antibodies† to Adenovirus Type 26										
		0	1	2	4	11	13	14	15	16	17	18
Unfractionated serum	HI‡	0	0	15	240	120	120	120	160	120	60	40
	NT§	0	0	++	++	++	++	++	++	++	++	++
"19S"‖	HI	0	0	0	0	0	0	0	0	0	0	0
	NT	0	0	0	0	0	0	0	0	0	0	0
"7S"‖	HI	0	0	4	64	48	32	48	32	32	24	12
	NT	0	0	0	++	++	++	++	++	+	+	++

* Adenovirus type 27 administered conjunctivally at weeks 0 and 11; type 26 administered at week 15.

† Geometric mean titers of samples from 2 volunteers.

‡ Hemagglutination-inhibition tests: 0 represents no activity at lowest dilution (1:10 for serum; 1:2 for immunoglobulin pools); reciprocals of sample dilutions are recorded.

§ Neutralization tests: 0 represents no activity at 1:2 dilution; + represents activity at 1:2 dilution only; ++ represents activity at ≥1:4 dilution.

‖ Immunoglobulin pools prepared by means of sucrose density gradient ultracentrifugation, as described in text.

antibody persisted for as long as 14 weeks after the administration of infectious virus, but no longer than 4 weeks after the inoculation of soluble antigens. Other workers have reported an early 19S followed by a later 7S antibody response to viral antigens administered to humans and to other animal species(5,6).

The reasons for the absence of a 19S IgM antibody response to experimental infection with adenovirus types 26 and 27 are not clear. Three major immunologic groups have recently been demonstrated among the adenoviruses, and types 26 and 27 have been classified in Group 2(3). When 32 volunteers, with and without pre-existing antibody to these adenoviral types, were inoculated with either type 26 or 27, they showed heterotypic HI antibody responses to 10 other serotypes within Group 2. It is tempting to speculate that this broad heterotypic antibody response indicated previous infection with one or more of these other serotypes. Thus, the antibody response to adenovirus type 27 in the present study may not have been a response to a primary stimulus, which would be expected to evoke 19S IgM antibody formation; it may have been a response to a reexposure to the same or related antigens, which would be expected to evoke mainly 7S IgG antibody(6). Heterotypic neutralization antibody to adenovirus type 4 found in serum from volunteers inoculated with type 1 soluble antigens has also been shown to be mainly of the IgG class(2). Indeed, other workers have suggested that 7S IgG antibodies exert a negative feedback effect on 19S IgM antibody synthesis(7).

The fact that adenovirus types 26 and 27 have not been found in association with illness in nature may be related in some way to the different immunoglobulin response evoked by these virus types, as compared to types 1 and 4, both of which are known to cause naturally-occurring illness. However, clinical illness was caused by experimental administration of

TABLE II. Immunoglobulin Distribution of Hemagglutination-Inhibition and Neutralization Antibodies After Gel Filtration of Serum from a Volunteer Challenged with Adenovirus Types 27 and 26.*

Weeks after first challenge →		Antibodies to Adenovirus Type 27										
		0	1	2	4	11	13	14	15	16	17	18
Unfractionated serum	HI†	0	0	10	80	20	40	10	ND§	40	40	ND
	NT‡	0	0	++	++	++	++	++	ND	++	++	ND
"IgM"‖	HI	0	0	0	0	0	0	0	0	0	0	0
	NT	0	0	0	0	0	0	0	0	0	0	0
"IgA"‖	HI	0	0	16	64	32	32	16	8	8	4	8
	NT	0	0	+	++	+	+	+	+	+	+	+
"IgG"‖	HI	0	0	4	32	16	4	16	16	16	4	8
	NT	0	0	0	++	+	+	+	+	+	+	+

Weeks after first challenge →		Antibodies to Adenovirus Type 26										
		0	1	2	4	11	13	14	15	16	17	18
Unfractionated serum	HI†	0	0	40	320	80	80	80	ND	80	10	ND
	NT‡	0	+	++	++	++	++	++	ND	++	++	ND
"IgM"‖	HI	0	0	0	0	0	0	0	0	0	0	0
	NT	0	0	0	0	0	0	0	0	0	0	0
"IgA"‖	HI	0	0	32	≥128	64	32	32	32	32	16	16
	NT	0	+	0	++	+	++	++	++	++	++	++
"IgG"‖	HI	0	0	16	≥128	64	64	32	32	64	8	16
	NT	0	+	+	++	++	++	++	++	++	++	++

* Adenovirus type 27 administered conjunctivally at weeks 0 and 11; type 26 administered at week 15.

† Hemagglutination-inhibition test: 0 represents no activity at lowest dilution (1:10 for serum; 1:2 for immunoglobulin pools); reciprocals of sample dilutions are recorded.

‡ Neutralization tests: 0 represents no activity at 1:2 dilution; + represents activity at 1:2 dilution only; ++ represents activity at ≥1:4 dilution.

§ Not done.

‖ Immunoglobulin pools prepared by means of gel filtration, as described in text.

types 26 and 27, and the antibody formed did protect against rechallenge and cross-challenge.

It is unlikely that protein losses during the fractionation procedures accounted for failure to detect low levels of 19S IgM antibody. While the mean yield of serum IgM concentrated in the "19S" pools prepared by density gradient ultracentrifugation was only 11.0%, 42.3% of serum IgM was concentrated in the "IgM" pools prepared by gel filtration, in the present study. The latter yield should have been adequate detect low levels of IgM antibody, as shown previously(2).

The present study has also shown that antibody titers in the "IgG" and "IgA" pools rose and fell with antibody titers in unfractionated serum and that there were no significant boosts in homotypic or heterotypic antibody titers when volunteers were re-challenged with adenovirus type 27 or cross-challenged with type 26. It should be noted that the "IgA" pools prepared by gel filtration in this study contained 58.6% IgA and 41.4% IgG; thus, the antibody activity in these pools cannot be assigned definitely to IgA alone. These antibody levels remained elevated for the 18 weeks of the study, and were associated with protection against illness when the subjects were rechallenged with type 27 at week 11 and challenged with type 26 at week 15(1). This suggests that durable protection against reinfection with adenovirus may be provided by IgG or IgA antibody, or both antibodies. However, it is also possible that an immune mechanism distinct from humoral antibodies may play an important or decisive role in protection from adenovirus infection and that the antibodies measured in this study develop in parallel with such a hypothetical protective mechanism.

Summary. Two volunteers were given ade-

novirus type 27 by conjunctival inoculation, followed in 3 months by a rechallenge with type 27, and a month later by a heterotypic cross-challenge with type 26. Sequential serum specimens were fractionated by density gradient ultracentrifugation or by gel filtration; pools containing a preponderance of IgM, IgA or IgG were prepared from each specimen; and homotypic and heterotypic HI and neutralization antibody titers were measured in each serum specimen and immunoglobulin pool.

No 19S IgM homotypic or heterotypic antibody activity was detected.

Titers of IgG and IgA antibody rose sharply 2 weeks after the initial inoculation, and the pattern of their appearance was much the same as the pattern of appearance of antibody in unfractionated serum. No significant boosts of antibody titers were demonstrable after rechallenge or cross-challenge. Antibody remained present in serum and in "IgG" and "IgA" pools for the 18-week duration of the study.

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1. Kasel, J. A., Evans, H. E., Spickard, A., Knight, V., Am. J. Hyg., 1963, v77, 265.

2. Lehrich, J. R., Kasel, J. A., Rossen, R. D., J. Immunol., in press.

3. Kasel, J. A., Banks, P. A., Wigand, R., Knight, V., Alling, D. W., Proc. Soc. Exp. Biol. and Med., 1965, v119, 1162.

4. Martin, R. G., Ames, B. N., J. Biol. Chem., 1961, v236, 1372.

5. Svehag, S.-E., Mandel, B., J. Exp. Med., 1964, v119, 21.

6. Uhr, J. W., Finkelstein, M. S., ibid., 1963, v117, 457.

7. Fink, C. W., Miller, W. E., Jr., Dorward, B., Lospalluto, J., J. Clin. Invest., 1962, v41, 1422.

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Secretion by Guinea Pig Gastric Mucosa *in vitro*.* (31614)

R. L. SHOEMAKER,† G. SACHS, AND B. I. HIRSCHOWITZ

Division of Gastroenterology, Department of Medicine, and Department of Physiology, University of Alabama Medical Center, Birmingham

The nature and activity of the pumps in the mammalian stomach have not been measured *in vitro*. Reports describing the properties of the secreting guinea pig mucosa(1), and of the *in vitro* cat and rat mucosa(2,3) seem to indicate that there are certain marked distinctions between the *in vitro* mammalian preparations.

This paper will therefore outline some of the characteristics of the guinea pig preparations, with particular emphasis on ionic requirements for H^+ and Cl^- transport.

Methods. Guinea pigs (*Cavia porcellus*) of approximately 800 g weight were killed by CO_2 narcosis and the stomach removed. The serosa and external muscle layers were dissected off and the mucosa mounted in a lucite chamber as previously described for *Rana pipiens*(4). The nutrient bathing solutions were of the following composition in mM: Na^+ 132, K^+ 6, Ca^{++} 1.0, Mg^{++} 1, Cl^- 115, HCO_3^- 25, HPO_4^{--} 1.0, glucose 20 and the secretory in mM: Na^+ 152, K^+ 6, Cl^- 158. The solutions were gassed by bubbling 95% O_2 and 5% CO_2. For ion substitution experiments, sulfate was substituted for chloride and choline was substituted for sodium. Measurements of hydrogen rate were by the pH stat method, transmembrane potential difference (P.D.) by calomel electrodes with renewable KCl junctions, and resistance by sending 10 microamps of current in either direction and the short-circuit current (I_{sc}) as the current required to reduce the P.D. to zero after 30 seconds.

Fluxes were determined using Cl^{36} or Na^{22}

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