rubin and BSP suggest that in the rat temperature has a greater effect on bile flow than on biliary excretion. This differential effect is more evident in the bilirubin studies carried out in mice. Here, while bile flow diminished with hypothermia, the biliary concentration of bilirubin was found to increase significantly. These observations tend to support the contention(1) that the formation of bile is a consequence of at least two separate mechanisms: one controlling the introduction of water and electrolytes; the other controlling the active secretion of specific substances into the bile.

Summary. The influence of temperature on the maximum biliary excretion of bilirubin and sulfobromophthalein was studied in anesthetized rats and mice. Experimental techniques commonly employed in maximum biliary excretion studies were shown to significantly alter normal thermoregulatory mechanisms. A decrease in body temperature significantly decreased the bilirubin transport maximum in both rats and mice. A loss of body temperature in the rat also produced a significant decrease in the BSP transport maximum. In both rats and mice bile flow showed a diminution corresponding with the decrease in rectal temperature. The results emphasize the importance of monitoring body temperature during the course of experiments employing maximum biliary excretion as an endpoint.

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Immunoglobulin Classes of Serum Neutralizing Antibody Formed in Response to Infection with Human A_2 Influenza Virus. (32081)

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Immunity to infection with influenza virus is not always directly related to the level of serum antibody(1-3). Following exposure to influenza virus, some individuals with low serum antibody titers are protected, while others with somewhat higher titers may become ill. Data obtained from volunteer studies suggest that antibody acquired by infection with antigenically related strains of A_2 virus may be less protective than antibody induced by the infecting virus strain(4).

The present work was undertaken to study the sequence of immunoglobulin classes of neutralizing antibodies to human influenza viruses formed in response to experimental infection of adult volunteers with a strain of A_2 virus.

Materials and methods. Serum specimens.

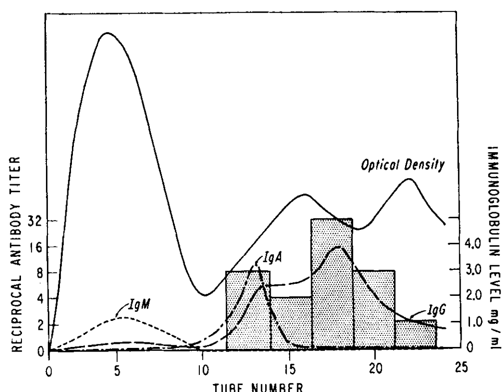

FIG. 1. Distribution of immunoglobulins and neutralizing antibody of serum following gel filtration on Sephadex G-200. Optical density was measured at 257 mμ in a flow-through spectrophotometer. Every 2 tubes were combined and concentrated 6 times by ultrafiltration. Broken lines represent immunoglobulin concentrations determined on each of the 2 tube concentrates. Bars represent neutralizing antibody titers determined on each of the 2 tube concentrates. Absence of bars indicates that no antibody activity was present.

Serum specimens were obtained from 5 adult male volunteers inoculated with approximately 10^5 50% tissue culture infectious doses of A_2/Bethesda/10/63 influenza virus. The care and supervision of these volunteers (5) and the method of virus inoculation have been previously reported(6). Each man was given a total of 2.5 ml of virus suspension; 1.5 ml was sprayed into the nose and mouth, and an additional 0.5 ml was dropped into each nostril. The volunteers were selected on the basis of low antibody titer to A_2/Bethesda/10/63 strain of virus (titers were 1:2 to 1:8). Four of the 5 volunteers developed typical febrile influenza, and the volunteer who did not become ill (volunteer #3) shed virus from the second through the sixth day following inoculation.

Gel filtration. The fractionation of serum was carried out using Sephadex G-200.* The effluent from each serum specimen was partitioned so that 3 fractions were obtained, each containing predominantly either IgM, IgA or IgG(7). The result of a typical fractionation is shown in Fig. 1. To form the 3 fractions, tubes 1-6 were combined into the "IgM" pool, tubes 11-13 combined into the "IgA" pool, and tubes 16-23 combined into the "IgG" pool.

Immunoglobulin assay. IgM, IgA and IgG concentrations were determined by the radial diffusion precipitin method in agar(8,9). Antisera were obtained commercially† and absorbed until they were specific for the corresponding immunoglobulin by immunoelectrophoresis against whole human serum at several test dilutions. Determinations of immunoglobulins were done in duplicate and comparative samples were assayed in the same plate.

Removal of IgA, IgG and IgM by precipitation with specific antisera. Antisera were prepared as described above. A mixture of 1 cc of the material to be absorbed and 0.1 cc of the appropriate antiserum was incubated in a water bath at 37°C for 30 minutes, then placed at 4°C for 2-4 days. After centrifugation, the same process was repeated on the supernatant using progressively smaller amounts of the antiserum, until no visible precipitate was obtained(10).

Hemadsorption-inhibition-neutralizing activity. Influenza antibody levels were determined by the neutralization test previously described(11). Titers on whole serum samples and eluate pools were measured in the same test. These specimens were tested against the inoculum A_2 influenza strain, and against A_2/Japan/305/57, A_1/FM1/47 and A/PR8/34 strains of virus. Thirty to 300 $TCID_{50}$ of tissue culture propagated virus were used in the tests.

Results. Homotypic antibody response. Antibody against the A_2/Bethesda/10/63 strain of virus was measured in sera obtained at weekly intervals. The results are shown in Table I and Fig. 2. In pre-inoculation specimens, antibody activity was detected in the "IgG" pool, and in 2 men there was measurable activity in the "IgA" pool. The level in these "IgA" pools was too low for absorption studies to determine whether this antibody activity was due to IgA or to IgG present in these pools. Two of 4 sera obtained at one week showed significant increases in titer from the pre-inoculation levels in whole serum and in the immunoglobulin pools.

* Pharmacia Fine Chemicals, Uppsala, Sweden.

† Hyland Laboratories, Los Angeles, Calif.

TABLE I. Neutralizing Antibody Titers in Whole Serum and Immunoglobulin Pools to Human Influenza Viruses in Adult Volunteers After Administration of A_2/Bethesda/10/63 Virus.

		Whole serum				"IgM" pool				"IgA" pool				"IgG" pool			
Volunteer	Day	Beth*	Jap	PR8	FM1	Beth	Jap	PR8	FM1	Beth	Jap	PR8	FM1	Beth	Jap	PR8	FM1
1	0	8†	32			0	8			0	16			2	16		
	7	32	64			2	8			2	16			4	4		
	14	256	256			8	16			32	32			64	64		
	28	128	128			8	16			16	128			64	128		
2	0	8	16	8	16	0	0	0	0	4	4	4	4	2	8	16	8
	14	2000	512	32	32	16	4	0	0	128	128	4	8	512	512	32	16
	28	512	512	32	32	0	2	0	0	32	8	8	4	256	256	32	4
3	0	8	64	8	4	0	2	0	0	4	32	4	0	8	32	4	0
	7	64	256	16	4	0	16	2	0	32	64	4	2	64	128	2	0
	14	≥4000	≥4000	16	16	8	16	0	0	128	512	4	0	1000	1000	4	0
	28	2000	1000	16	16	2	8	2	0	32	32	2	0	1000	2000	8	2
4	0	8	32	2	2	0	0	0	0	0	4	4	0	2	8	4	0
	7	8	32	16	4	0	0	0	0	2	4	2	0	4	16	4	0
	16	2000	2000	16	8	64	64	0	0	1000	256	4	2	1000	1000	16	0
5	0	2	4			0	0			0	0			0	0		
	7	4	8			0	0			0	0			0	2		
	20	512	512			4	4			32	32			128	128		

* Beth = A_2/Bethesda/10/63. Jap = A_2/Japan/305/57. PR8 = A/PR8/34. FM1 = A_1/FM1/47.
† Expressed as reciprocal of sample dilution.

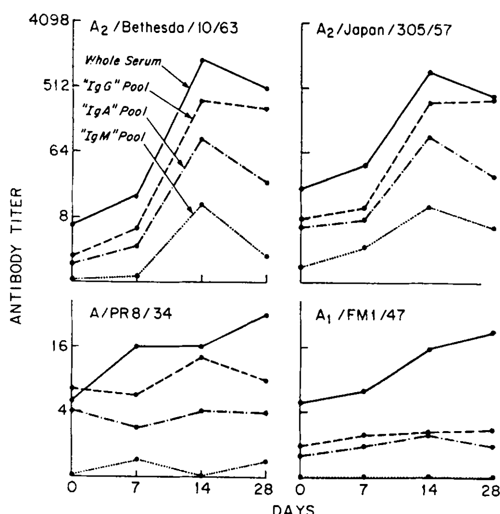

FIG. 2. Geometric mean neutralizing antibody titer of whole serum and immunoglobulin pools of the 5 volunteers. Titers are expressed as the reciprocal of the dilutions.

By 14 days the serum of all men contained maximum antibody levels. Most of the activity was in the "IgG" pools, although some antibody was present in the "IgM" pools and slightly more in the "IgA" pools. Moreover, absorption studies indicated that the antibody activity in the "IgA" and "IgM" pools was probably due to the presence of IgG in these pools (Tables II and III). By 28 days the titers had decreased slightly.

Heterotypic antibody response. Pre-inoculation antibody levels to A_2/Japan/305/57 virus were slightly higher than levels to the challenge virus. After inoculation the antibody responses to both viruses were nearly identical. For example, in volunteer #3, the whole serum titers against the challenge virus increased from 1:8 to 1:64 to 1:4000 and the "IgG" pool titers increased from 1:8 to 1:64 to 1:1000 at 0, 7, and 14 days, respectively. Against A_2/Japan/305/57 virus the whole serum titers in the same volunteer increased from 1:64 to 1:256 to 1:4000 and the "IgG" titers increased from 1:32 to 1:128 to 1:1000.

In response to administration of the inoculum virus, there was a 2- to 8-fold rise in antibody titer to A/PR8 virus. The rise occurred in parallel to that noted against the inoculated virus, and the antibody, both prior to and after inoculation, was located primarily in the "IgG" pool.

There was a similar rise in antibody titer against the A_1/FM1 strain of virus.

Removal of IgA and IgG by precipitation with specific antisera (Tables II and III). The "IgA" pool prepared from serum obtained on day 14 from volunteer #3 was selected for absorption studies because of its relatively high antibody titer against A_2/Bethesda/10/63 virus. Removal of over 80% of the IgA, as determined by the radial diffusion precipitin method, resulted in no change in titer. However, removal of approximately 60% of the IgG resulted in a decrease in titer from 1:128 to 1:32.

The "IgM" pool prepared from serum obtained on day 16 from volunteer #4 was similarly selected for absorption studies. Removal of over 90% of the IgM resulted in no change in titer. However, removal of approximately 70% of the IgG resulted in a decrease in titer from 1:64 to 1:4.

Discussion. From the data presented it appears that the serum antibody formed in human volunteers in response to experimental A_2 influenza virus infection is IgG immunoglobulin. In most other systems studied, including other viral infections, antibody was detected first in the IgM and later in the IgG immunoglobulins(12-16). In mice, injection of emulsified inactivated A/PR8 influenza

TABLE II. Antibody Titer to A_2/Bethesda/10/63 Virus Following Absorption of "IgA" Pool.

	Titer	Immunoglobulin concentration* IgA	IgG
"IgA" pool	1:128	2.1	1.2
"IgA" pool + anti-IgG	1:32	2.1	.5
"IgA" pool + anti-IgA	1:128	.4	1.3

* Expressed as mg/ml.

TABLE III. Antibody Titer to A_2/Bethesda/10/63 Virus Following Absorption of "IgM" Pool.

	Titer	Immunoglobulin concentration* IgM	IgG
"IgM" pool	1:64	1.15	.32
"IgM" pool + anti-IgM	1:64	<.10	.50
"IgM" pool + anti-IgG	1:4	.86	.10

* Expressed as mg/ml.

virus vaccine resulted first in the appearance of IgM hemagglutination-inhibiting antibody, as shown by sucrose gradient ultracentrifugation and treatment with 2-mercaptoethanol (12). Peak IgM antibody titers occurred at day 4 and this IgM antibody accounted for most of the HI activity through day 7 and persisted for at least 34 days. In rabbits injected with poliovirus by various routes IgM antibody appeared first and reached a maximum on day 4(13). If the inoculum was large enough IgG antibody appeared later. Similar results were obtained in humans with the natural infection or following oral attenuated virus vaccination(13). In mice given actinophage, in early sera the neutralizing activity was associated with IgM; the late sera, with IgG(14).

In patients with primary and recurrent herpes simplex infection, the initial antibody response was characterized by an increase in the IgM titer, shown by precipitation of IgM antibody by rabbit anti-gamma M (15). The initial response was followed after 21 days by a predominance of IgA and IgG antibodies. It has been shown that volunteers inoculated with infectious adenovirus 1 or 4 or with preparations of adenoviral hexon or fiber antigens prepared from these serotypes developed IgM antibody which appeared simultaneously with IgG and IgA antibody (7). However, after inoculation with live adenovirus types 26 and 27, no IgM HI or neutralizing antibodies could be detected by either sucrose density ultracentrifugation or Sephadex G-200 gel filtration(16).

In newborn infants immunization with Salmonella vaccines resulted in production of 17-20 S agglutinins as early as 7 days and 7 S agglutinins by the 30th to 40th day(17). In adults macroglobulin agglutinins appeared on day 4 or 5, and 7 S agglutinins 3 or 4 days later.

Antibody activity against influenza A viruses in the currently reported study was not detected in the IgM class of immunoglobulins. The earliest serum specimens were collected 7 days after inoculation and IgM antibody could have disappeared by that time. However, in the studies cited above, IgM antibody persisted well past the seventh day. Furthermore, the half-life of IgM precludes the possibility that significant amounts of IgM antibody were formed and then destroyed by the seventh day(18). A second explanation for the lack of IgM antibody may be that it develops only after a primary exposure to an antigen. Since the volunteers in this study had detectable pre-inoculation antibody to A_2/Bethesda/10/63 virus they may have been infected previously with this or a related A_2 virus strain. In measles immune persons, injection of inactivated measles vaccine resulted in a rise in 7 S antibody titers, but no rise in 19 S antibody titers (19). However, it has been shown that IgM is the first class of antibody to appear after repeated challenges with poliovirus(13) and in recurrent herpes simplex infections(15). In rabbits, which were given poliovirus by various routes of inoculation, the IgM secondary response was no different from the primary response, even though IgG antibody was present at the time of the secondary response (13). A third explanation for the failure to elicit an early IgM response could be related to the route of inoculation. However, serum IgM antibody developed in a volunteer inoculated *via* the respiratory tract with a strain of rhinovirus(20), and in 3 volunteers inoculated conjunctivally with strains of adenovirus(7).

Other studies have shown that IgA neutralizing and HI antibody appear in nasal secretions of volunteers infected with A_2 influenza virus(21). Maximum levels of nasal secretion antibody were present by day 13 in most instances. The results of the present study show no appreciable IgA serum neutralizing antibody. Of special interest was volunteer #1 who, in the study reported above (21), had the highest level of nasal secretion antibody and in whom we could not find serum IgA influenzal antibody.

Brown *et al* reported that after an epidemic of A_2/Taiwan/1/64 12 of 53 isolated natives without serum antibody to A, A_1 or A_2 influenza viruses developed antibody to either one or both A/PR8 and A_1/Ann Arbor/1/57 (22). The few who had low antibody titers to A and A_1 strains had 4-fold or greater rises following the epidemic. The results of

the present study are in accord with their observation that infection with an A_2 strain stimulates antibody production against A and A_1 strains.

Summary. In volunteers the sequential appearance of the immunoglobulin classes of neutralizing antibody formed as a result of infection with A_2/Bethesda/10/63 influenza was studied. The antibody appeared in the IgG class and no significant antibody in the IgM or IgA classes was found. It was particularly notable that no IgM antibody could be detected 7 days after inoculation. The pattern of antibody against A_2/Japan/305/57 was nearly identical to that formed against the inoculum strain. Antibody rises were also noted against A/PR8 and A_1/FM1 influenza viruses.

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Lipid Mobilizer Hormone in Triton Hyperlipemia.* (32082)

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A lipid-mobilizing hormone (LM) was discovered in the plasma of man and other species by Seifter and Baeder(1) in 1954. These workers later isolated LM from the posterior pituitary gland of hogs(2) and demonstrated that the plasma content of LM is greatly increased by administration of cortisone and by subjecting animals to cold or to the nephrotic syndrome(1,3). Lipovetskii reported confirmatory findings for the presence of LM in human plasma(4) as have Kadas and Nagy(5). The latter authors also demonstrated that LM was derived from the posterior pituitary in further confirmation of the reports of Seifter and Baeder.

LM exerts two major effects: (1) it mobilizes triglycerides from the omental and mesenteric depots, and (2) it inhibits the delactescent action of heparin-clearing factor *in vitro*. Extensive studies have shown LM

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