amounts of serotonin, norepinephrine and dopamine. This finding suggests that dietary (exogenous) precursors can increase the urinary excretion of 5-OH indole compounds.

Summary. 1. Banana ingestion (12 g/kg/24 hr) by normal adults produced an average 2-fold increase in urinary excretion of 5-OH indoles. This was not of such magnitude as to suggest an erroneous diagnosis of malignant carcinoid. 2. Collection of 24-hour urine specimens and use of a quantitative test are advocated over collection of random urine specimens. Positive screening tests should be checked by a quantitative method.

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Hemagglutination with Adenoviruses. Nature of Viral Antigen. (24089)

EUGENE ROSS* AND HAROLD S. GINSBERG

Departments of Preventive Medicine and Medicine, Western Reserve University, and University Hospitals, Cleveland, O.

It has been demonstrated that antigens can be adsorbed to tannic acid-treated erythrocytes so that the red blood cells agglutinate when mixed with specific antiserum(1,2,3). An attempt therefore was made to apply this technic to a study of the antigens of adenoviruses and the antibodies directed against these viral materials. Since the completion of this investigation it has been reported that antibodies directed against adenoviruses(4), herpes simplex(5), and poliomyelitis(6) viruses can be measured by use of tannic acid-treated sheep erythrocytes. The study to be reported confirms the data presented by Friedman and Bennett(4), and indicates that a modified hemagglutination technic can measure not only the adenovirus group antigen but also type-specific antibodies in approximately 50% of the sera tested. In addition, identification of the major antigen which adsorbs to tannic acid-treated erythrocytes will be described.

Materials and methods. Tissue culture. Strain HeLa cells (Gey) derived from an epidermoid carcinoma of the uterus were employed. The methods used for serial propagation and preparation of tube cultures have been described(7). The nutrient fluid consisted of 40% human serum and 60% Hanks' balanced salt solution or Eagle's basal medium supplemented with 10% human serum. *Viruses.* Adenoviruses types 1, 2, 3 and 4 were grown in HeLa cells using a maintenance mixture consisting of Scherer's amino acid-vitamin mixture (MS) 67.5%, chicken serum 7.5%, and tryptose phosphate broth 25% (8). Virus suspensions were prepared by freezing and thawing infected cells 6 times following which cellular debris was removed by centrifugation(7). Viruses were stored in sealed glass ampules at –70°C or in screw cap tubes at –30°C. *Hemagglutination (HA) test.* A modification of the method reported by Stavitsky(3) was used. Chicken erythrocytes were employed because a more distinct endpoint was obtained with these cells than with sheep or rabbit red blood cells. Erythrocytes from white leghorns were obtained weekly and stored in Alsever's solution at 4°C. Before use, cells were washed 3 times with

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0.85% NaCl buffered at pH 7.1 with 0.15 M phosphate. This salt solution will henceforth be called saline. A 2.5% suspension of cells was prepared and standardized at a reading of 500 with a 540 mμ filter in a Klett colorimeter. The 2.5% suspension of chicken rbc was incubated with an equal volume of a 1:20,000 solution of tannic acid at 37° for 10 minutes. The treated cells were washed once with an equal volume of saline and then resuspended to 2.5% in saline. Tanned rbc were prepared daily. Virus suspension prepared as described was diluted with an equal volume of saline in order to obtain results compatible with both economy and antibody titers high enough to be interpreted. Adsorption of viral antigen to tanned rbc ("sensitization") was carried out by mixing 1 volume of cells, 1 volume of diluted virus suspension and 4 volumes of 0.15 M phosphate buffer at pH 6.4. Tanned rbc and virus were incubated at room temperature for 10 minutes. Sensitized cells were centrifuged at 1000 rpm for 5 minutes, washed twice with a 1:100 normal rabbit serum solution in saline, and resuspended in 1:100 normal rabbit serum as a 1% suspension. Equal volumes (0.3cc) of the suspension of virus-treated cells and serial dilutions of serum were incubated at room temperature for exactly 60 minutes and read for hemagglutination. The last tube in which definite hemagglutination was observed was considered as the endpoint. All titers are expressed as the reciprocal of the final dilution of serum. *Sera.* Acute and convalescent human sera from cases of proved adenovirus infections were centrifuged free of particulate matter and heated at 56°C for 30 minutes. Serial 2-fold serum dilutions were prepared in 1:100 normal rabbit serum. *Serum neutralization and virus infectivity titrations* were done exactly as described previously(7). A standard *complement-fixation* titration using 2 units of complement was employed. The highest dilution of serum which caused complete fixation of complement was taken as the endpoint. The titer is expressed as the reciprocal of the final dilution of serum in the initial phase of the reaction.

Results. The technic for adsorption of antigen to tannic acid-treated red blood cells was shown to be applicable to adenoviruses. The major modification in the technic described by Stavitsky(3) was the use of chicken erythrocytes to obtain sharp and reproducible endpoints readily and after a short period of incubation (60 minutes). Results of experiments which investigated conditions for sensitization of red blood cells with adenovirus antigen, heat stability of the antigen, and quantity of antigen necessary to obtain maximum hemagglutination confirmed the studies reported by Friedman and Bennett (4) and therefore will not be described here. Moreover, as their report indicates, it demonstrated an increase in titer of antibodies in sera of patients convalescent from adenovirus infections. By technics employed in the initial part of this study and by previous workers, however, antibody titer in the acute phase serum was occasionally high, and an antibody rise could be detected with 2 or more types of virus. That is, the antibody demonstrated by the hemagglutination method, just as with complement-fixation titration, appeared to be group rather than type specific.

Experiments were therefore designed to develop a method by which type-specific antibodies could be measured, on the assumption that, if the heterologous viruses were pooled and used as "blocking antigens" to react with non-type-specific antibodies, the type-specific antibody could be detected. The "blocking antigen" pool consisted of equal volumes of each of the 3 heterologous viruses. To 0.15 cc of each serial dilution of the serum to be tested was added 0.15 cc of the "antigen pool." The serum dilution-virus pool mixtures were incubated at 37°C for 30 minutes after which there was added to each tube 0.3 cc of a suspension of tanned rbc previously reacted with the type of virus for which antibodies were to be measured. The results of an experiment with and without the use of "blocking antigens" are summarized in Table I.

By neutralization titrations the sera being tested showed a rise in type 3 antibodies, a questionable rise in type 2 antibodies, and no increase in type-specific antibodies to type 1 or 4 virus. Without the use of the pools of

TABLE I. Effect of Heterologous Virus Pools as "Blocking Antigens" on Hemagglutination Antibody Titers.

Antigen		HA antibody titer of serum			
		No "blocking antigens"		With "blocking antigens"	
Serum mixed with virus pool	Virus adsorbed to tanned RBC	Acute	Conval.	Acute	Conval.
Types 2, 3, 4	Type 1	>4096	>4096	>5120	>5120
1, 3, 4	2	4096	"	< 20	20
1, 2, 4	3	2048	4096	"	>5120
1, 2, 3	4	"	"	1280	1280
—	Normal HeLa	<8	<8	<8	<8

heterologous viruses it was not possible to determine the specific type of adenovirus responsible for the infection. With "blocking" viral antigens a clear cut rise in type 3 antibody was measured as well as a slight increase in type 2 antibodies. These results were similar to those obtained by neutralization titrations. Although results were not always as definitive as in this experiment, the data implied that pre-incubation with "blocking antigens" was a useful procedure in order to detect a type-specific reaction.

To determine the specificity of the hemagglutination reaction, the results of hemagglutination, neutralization, and complement-fixation titrations with the same sera were compared. To quantitate antibodies by the hemagglutination technic, "blocking" heterologous viruses were employed. Eight pairs of sera were tested. In 4 pairs the antibody responses to infection as measured by hemagglutination were identical to those obtained with neutralization. In the remaining 4 pairs of sera, results of hemagglutination titrations were similar to those demonstrated with the complement-fixation technic in that the specific type of virus which caused infection could not be determined; rather an increase in antibody titer for 2 or more types was observed. The reasons for the inability of blocking antigens to eliminate non-specific reactions regularly have not been ascertained. Results of antibody titrations with 2 pairs of sera, selected to illustrate range of variation noted, are summarized in Table II. A type-specific rise in hemagglutination antibodies in serum pair II closely mimics the results obtained by neutralization titration. It is interesting to note that although there is a high titer of type 1 antibodies in the acute phase serum, the specific etiology of the infection could be demonstrated.

Identification of viral antigen adsorbed to tanned rbc. To determine whether the antigenic material which adsorbed to chicken erythrocytes was part of the viral particle *per se* or was a smaller antigenic particle, the viral suspensions were centrifuged at 105,400 x g for 60 minutes in a Spinco Model L preparative centrifuge. The upper one-half of the supernate was removed carefully, the remaining fluid discarded, and the sediment resuspended to the original volume in Hanks' balanced salt solution. The results of infectivity titrations and hemagglutination antibody titrations done with the original viral suspensions, supernates, and resuspended sediments of the type 1 and 3 viruses are summarized in Table III. The supernatant fluids contained less than 1% of the infectious virus in the uncentrifuged material and the sediment comprised 10 to 32% of the total infectious virus. Despite the relatively small amount of infectious agent remaining in the supernate, the hemagglutination antibody titer measured by rbc sensitized with this fraction was similar to that obtained with rbc mixed with the original viral suspension. Erythrocytes treated with the resuspended sediment, however, yielded considerably lower antibody titers. The results reported by Friedman and Bennett(4) and here confirmed indicated that antibody titer obtained by hemagglutination titration was directly proportional to quantity of virus employed to sensitize rbc. These quantitative considerations indicate that each supernate used in the experiments described in Table III contained

TABLE II. Comparison of Antibody Titers as Measured by Hemagglutination, Complement-Fixation and Neutralization Titrations.

Serum	Antibody titration method	Antibody titer — Type virus 1	2	3	4	HeLa control
I acute *	Hemagglutination	32	2048	8	8	16
conval.		2048	"	512	512	"
I acute	Neutralization	<8	<8	8	<8	
conval.		"	8	512	8	
I acute	Complement-fixation	"	8	<8	<8	
conval.		128	256	128	128	
II acute	Hemagglutination	4096	32	32	32	16
conval.		"	"	512	"	"
II acute	Neutralization	<8	<8	<8	<8	
conval.		"	"	32	"	
II acute	Complement-fixation	16	8	<8	"	
conval.		128	256	256	256	

* Acute and convalescent phase.

as much antigenic mass adsorbable onto tanned rbc as the uncentrifuged specimen, and significantly more rbc-adsorbable antigen than was present in the sediment fraction although the latter material had 10 to 30 times more infectious virus. Similar results were obtained in experiments carried out with types 2 and 4 adenoviruses. These data clearly imply that the major antigen responsible for the hemagglutination of rbc when mixed with specific antibody is viral material which is separable from the infectious viral particle and probably smaller in size than the infectious unit.

Discussion. Adsorption of antigens to tannic acid-treated erythrocytes, first demonstrated with bacterial products(1), has been applied to adenoviruses(4), herpes simplex (5), and poliomyelitis(6) viruses. Agglutination of the treated and sensitized rbc by specific antibodies is the indicator by which antigen is detected and also serves as a technic for quantitation of antibodies. The evidence here presented confirms previous reports(4) that this technic does not measure adenovirus type-specific antibody. Rather antibodies to the group antigen, as detected by complement-fixation test, are titrated by the hemagglutination technic. A preliminary reaction of serum with a pool of heterologous viruses, however, permitted utilization of the hemagglutination technic to measure type-specific antibodies in 4 of 8 sera. It may be possible to obtain type-specific antibody rises in more sera if the pool of heterologous antigens contains more of the known types of adenoviruses. The data imply that antibody detected by hemagglutination titration is directed chiefly against an antigen of the homologous virus. Although comparable experiments have not been done by complement-fixation titrations, some evidence suggests a similar antigen may be involved in this technic(9).

TABLE III. Effect of High Speed Centrifugation* on the Hemagglutination Antigen and the Infectious Virus.

Virus type	Material tested	Infectivity titer (log)	HA antibody titer†
1	Original	−5.0	128
	Supernate	−2.5	128
	Sediment	−4.0	16
3	Original	−3.0	512
	Supernate	−1.0	1024
	Sediment	−2.5	16

* Viral suspensions centrifuged at 105,400 × g for 60 min.

† Dilutions of a human convalescent serum were mixed with tanned red blood cells previously treated with the viral material to be tested.

High speed centrifugation of adenovirus-infected culture fluids demonstrated that the antigen which adsorbs to tanned chicken rbc is separable from the viral particle and is soluble. This evidence further suggests that the antigen may be similar to that responsible for complement-fixation. The hemagglutination technic offers not only another means of measuring viral antibodies, but also intro-

duces a sensitive method for detecting small quantities of viral antigens and for studying differences in antigens related to a single virus.

Summary. A rise in antibodies to types 1-4 of adenoviruses during illness can be detected in human serum by employing hemagglutination technics. Although the original method described does not measure an increase in antibodies directed against a single type of virus, type-specific antibodies can be titrated if serum is mixed with a pool of heterologous viruses before tanned rbc treated with homologous virus are added. This modification measured type-specific antibodies solely in 4 of the 8 sera tested. The antigen adsorbed to tannic acid-treated rbc is probably not the infectious viral particle and is separable from it by high speed centrifugation.

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Variation and Hemolysin Production in Relation to Virulence of *Leptospira pomona.** (24090)

D. C. BAUER AND E. V. MORSE†
Department of Microbiology and Public Health, Michigan State University, East Lansing

Properties of leptospirae which contribute to their virulence have not been well established. Fukushima and Hosoya(1) and Higuchi(2) demonstrated substances toxic for guinea pigs in cultures of *Leptospira icterohemorrhagiae* maintained anaerobically or *in vacuo*. Stavitsky(3) was unable to verify these findings. Fibrinolysin, leukocidin, and coagulase could not be demonstrated in filtrates of leptospiral cultures(4). Volland and Brede reported(17) that hyaluronidase was produced by leptospirae *in vitro*. A soluble hemolysin has been demonstrated in cultures(5,6) and it has been suggested that the hemolytic activity of leptospirae is an attribute of a toxin(5). Pathogenic leptospirae decrease in virulence upon continual transfer in culture media(8). Presumably reduction in virulence of a given culture is due to *in vitro* development of avirulent variants. The present investigations concern(1) changes in virulence of *Leptospira pomona* for hamsters following passage in media, and (2) *in vitro* hemolysin production as correlated to virulence.

Materials and methods. Virulence determinations. Two strains of *L. pomona* were employed. Strain W had been isolated from bovine urine(9) and maintained by serial passage in weanling guinea pigs. Although this strain was not lethal for hamsters, it was pathogenic for cattle, swine, sheep, goats, and dogs. Strain O had been maintained in continuous hamster and guinea pig passage since its isolation from porcine urine. Strain O killed hamsters and was pathogenic for sheep, swine, and dogs. The first medium passage of Strain W was obtained as blood culture from a sheep which had been inoculated with infected guinea pig blood. Medium passages of Strain O were initiated by culturing blood from an infected guinea pig. Maximum growth of initial hemocultures was obtained after approximately 30 days. Chang's fluid medium(10),

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† Present address: Veterinary Med. Research Inst., Iowa State College, Ames.