The low serum antitryptic activity observed after hypophysectomy when compared with adrenalectomy would indicate that in addition to the pituitary-adrenal cortex axis other endocrine systems may be involved in the physiological control of the fibrinolytic system.

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Received April 14, 1958. P.S.E.B.M., 1958, v98.

Increased Avidity of Antibody for Baltimore 1957 Strains of Asian Influenza Virus (P-Q-R Variation)* (24043)

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Influenza A viruses isolated in Asia during early stages of the 1957 pandemic appeared to have poor antigenic potency. Serum antibody titers of patients convalescent from Asian influenza were lower than anticipated (1), and considerable doubt was cast on the efficacy of the new vaccines because they seemingly failed to induce significant antibody responses. Alternatively, these findings could be explained by insensitivity of viral antigens used to measure antibody titers. It had previously been shown that antigenically related influenza A' viruses often differed markedly in their capacity to combine with specific antibody, a phenomenon known as the P-Q-R variation(3,4). That Asian influenza viruses might undergo the same transformation was implied by Meyer, *et al.*(1) who noted that "certain of the early passage egg fluids of strain A-Japan-305-57 were not readily inhibited by homologous immune serum and appeared to be in Q phase." This supposition is confirmed by serologic studies herein reported, which demonstrate that influenza viruses isolated from patients in Baltimore during autumn of 1957 were in the R phase, although indistinguishable antigenically from the prototype Asian influenza strain A-Japan-305-57.

Materials and methods. Viruses. The prototype Asian strain of influenza A virus, A-Japan-305-57, was obtained from Walter Reed Army Medical Research Institute as lyophilized allantoic fluid of 5th consecutive egg passage. Eight additional strains of virus used were isolated in Oct. 1957 from patients at Johns Hopkins Hospital and are designated by convention as A-Baltimore-1001-57 to A-Baltimore-1008-57. Six of these viruses were recovered from throat washings of patients with clinical manifestations of acute influenza and the remaining 2 from lung tissue of patients who died several days after onset of illness. Penicillin and streptomycin were added to the throat washings or 10% suspensions of lung tissue and 0.1 ml of each material was inoculated into amniotic cavity of chicken embryos 8 days of age. After incubation for an additional 5 days at 37°C the embryos were chilled, amniotic fluids harvested and the presence of virus determined by capacity of fluids to agglutinate an equal volume of 1% guinea pig erythrocytes. Each virus isolate was passaged an additional 1-3 times by the allantoic route in 10 or 11-day-old chicken

* Supported by grant from Nat. Cancer Inst., U.S.P.H.S., and contract with U. S. Army Chemical Corps, Fort Detrick, Md.

embryos and the final virus suspension was stored at –60°C prior to use. A-Japan-305-57 virus was studied after it had undergone a total of 9 allantoic passages. *Immune sera.* The Communicable Disease Center of the U.S. Public Health Service kindly supplied 2 standard preparations of chicken antiserum, anti-A-Japan-305-57 and anti-A-Denver-1-57 (a recent A′ strain). Additional immune sera were prepared in this laboratory by intraperitoneal injection of chickens with A-Japan-305-57 or A-Baltimore-1001-57 viruses. Each chicken received approximately 320-400 CCA doses of a virus suspension on each of 3 successive days and was bled 8 days after last injection. The Maryland State Dept. of Health generously furnished paired serum specimens from men vaccinated with commercial monovalent Asian influenza vaccine. Paired sera were also obtained from patients admitted to Johns Hopkins Hospital with clinical diagnosis of influenza. Sera from vaccinated individuals were received after they had been inactivated at 56°C for 30 min.: all other human and chicken sera were unheated and stored in frozen state without addition of preservatives. None of the sera was treated with cholera filtrate or periodate. *Hemagglutination titrations* were performed by modification of the Salk(2) pattern test, in which the endpoint was read as partial (50%) agglutination of 0.5% chicken erythrocytes in final volume of 0.5 ml. In the *hemagglutination-inhibition (HI) tests* serial 2-fold dilutions of serum were made in 0.25 ml of 1% chicken erythrocytes and 5 agglutinating doses of virus were added to each tube to give final volume of 0.5 ml. The tests were read after incubation at 4°C for 30 minutes (which gave the clearest pattern). HI titers are expressed as original dilution of serum producing partial (50%) inhibition of hemagglutination. *Neutralization tests* were performed in chicken embryos with 1% normal and immune chicken sera heated at 56°C for 30 minutes. Higher concentrations of immune serum completely neutralized infectivity of one of the strains. Serial 10-fold dilutions of virus were made in normal or immune serum, or broth-saline solution. After incubation of the virus-serum mixtures for 1 hour at room temperature, they were inoculated by allantoic route into groups of 6 eggs. Allantoic fluids were harvested 44 hours later, and the presence of virus determined by agglutination of 1% chicken erythrocytes. The 50% egg infective dose (EID_{50}) was calculated by the method of Reed and Muench.

TABLE I. HI Antibody Titers of C.D.C. Reference Chicken Antisera.

Virus strain	Source	Antisera: A-Japan-305-57 (Asian)	Antisera: A-Denver-1-57 (A′)
A-Japan-305-57	Prototype	80*	<10
A-Balt. -1001-"	Throat	640	<10
-1002-"	"	160	"
-1003-"	Lung	240	< 5
-1004-"	Throat	160	<10
-1005-"	Lung	"	"
-1006-"	Throat	320	10
-1007-"	"	"	<10
-1008-"	"	160	20

* Reciprocal.

Results. Serologic identification of Baltimore viruses. The 8 strains of virus isolated during these studies were identified as Asian serotypes by HI tests performed with reference C.D.C. chicken antisera (Table I). As noted, anti-A-Japan-305-57 antiserum inhibited hemagglutinating activity of all 8 Baltimore viruses at dilutions of 1:160 or greater, whereas antiserum prepared against the A′ Denver strain gave HI titers of 1:20 or less. Non-specific inhibitors rather than antibody may have accounted for the slight inhibitory effect of anti-A-Denver-1-57 serum against 2 of the Baltimore viruses. However, the most striking finding was that anti-A-Japan-305-57 antiserum was invariably a more effective inhibitor of the Baltimore viruses than of the homologous strain.

Cross hemagglutination - inhibition tests with Japanese and Baltimore viruses and their respective antisera. Further studies were carried out to characterize the antigenic relationships of viruses isolated in Japan and Baltimore and to determine their relative capacities to stimulate antibody production. Three chickens were immunized with an allantoic fluid suspension of A-Japan-305-57 virus and 3 others with A-Baltimore-1001-57 virus, each

TABLE II. HI Titers of Antisera from Chickens Immunized with Equivalent Amounts of Japanese or Baltimore Viruses.

Virus strain	Antiserum: Anti-A-Japan-305-57 C81*	C82	C83	Anti-A-Balt.-1001-57 C84	C85	C86
A-Japan- 305-57	60†	160	160	120	240	60
A-Balt. -1001- "	1920	640	1280	2560	1920	2560
-1003- "	640	1280	480	960	2560	1920

* C81 = Chicken No. 81.
† Reciprocal of serum dilution.

of the 6 animals receiving equivalent amounts of viral hemagglutinin on the same dosage schedule. Sera prepared from immunized chickens were tested individually for their HI antibody content against Japanese and 2 Baltimore viruses. Table II demonstrates that high titers of HI antibody were produced against both strains of Baltimore virus, regardless of whether chickens were immunized with Japanese or Baltimore virus. Similarly, both types of antisera inhibited A-Japan-305-57 virus to the same degree. It appears, therefore, that Japanese and Baltimore viruses have similar antigenic structures and equivalent immunizing potency in chickens. However, it is readily apparent that the hemagglutinating activity of A-Japan-305-57 virus was invariably less well inhibited by either homologous or heterologous antisera than were hemagglutinins of the Baltimore viruses. Therefore, the Japanese strain was in the Q phase, differing serologically from 2 Baltimore strains only in its relative insusceptibility to antibody.

Increased susceptibility of Baltimore virus to neutralization by immune serum. Infectivity of Baltimore virus for chicken embryos was more readily neutralized by anti-A-Japan-305-57 serum than was the infectivity of the homologous virus. The titers of A-Japan-305-57 virus diluted in broth-saline solution, 1% normal chicken serum and 1% immune serum were $10^{7.5}$, $10^{6.3}$, and $10^{5.2}$ EID_{50}, respectively. Corresponding determinations of infectivity of Baltimore-1003-57 virus diluted in broth-saline solution, normal serum and immune serum were $10^{5.7}$, $10^{5.9}$, and $10^{2.2}$ EID_{50}, respectively. Therefore, the neutralization index for A-Japan-305-57 virus, calculated on basis of its infectivity in 1% normal and immune chicken serum, was 12, whereas neutralization index for A-Baltimore-1003-57 virus was 5000. Thus, the Baltimore virus, compared with the prototype Japanese strain, exhibits greater sensitivity to neutralization of infectivity, as well as greater sensitivity to inhibition of hemagglutination by immune serum.

Paradoxical serologic response of individuals vaccinated with A-Japan-305-57 virus. Paired sera were obtained from men immunized with a single intradermal injection of 0.1 ml of formalinized vaccine prepared from A-Japan-305-57 virus. This vaccination program had been instituted the month prior to onset of the Baltimore epidemic. It is highly unlikely, therefore, that these individuals had been exposed to active infection with Asian influenza virus before post-vaccination sera were obtained. The antibody content of serum collected on the day of vaccination and 14-22 days later was determined by HI tests using antigens prepared from A-Japan-305-57 and 2 strains of Baltimore virus. Representative data presented in Table III demonstrate that no antibodies could be detected against the homologous strain of virus. Slight anti-hemagglutinating activity of pre-vaccination sera against the Baltimore viruses can be attributed to nonspecific inhibitor. Notwithstanding, each vaccinated individual showed a definite rise in antibody titer against the heterologous viruses. It is evident, therefore, that HI tests with R phase Baltimore viruses are more sensitive indicators of immunologic response than are tests with the prototype

TABLE III. HI Antibody Response to Intradermal Vaccination with A-Japan-305-57 Virus.

Virus strain	Human sera: No. 30244 Pre*	Post*	No. 30563 Pre	Post	No. 30566 Pre	Post
A-Japan-305-57	0†	0	0	0	0	0
A-Balt.-1001-57	0	80	10	80	20	80
1003-57	0	20	30	160	40	160

* Pre- or post-vaccination.
† 0 = <5 (reciprocal).

TABLE IV. HI Antibody Response to Infection during the 1957 Baltimore Epidemic of Influenza.

	Patients' sera							
	P. MeC.		S. W.		H. B.		E. P.	
Virus strain	Acute	Conv.	Acute	Conv.	Acute	Conv.	Acute	Conv.
A-Japan-305-57	<10*	80	<10	40	<40	80	<10	<10
A-Balt.-1003-57	30	320	"	80	"	320	"	40

* Reciprocal of serum dilution.

Asian strain in the Q phase.

HI antibody response to infection. Serum was obtained from unvaccinated patients within 48 hours of onset of influenza and 2-3 weeks later. These paired sera were tested against both A-Japan-305-57 and A-Baltimore-1003-57 viruses. Representative data on 4 patients are shown in Table IV to illustrate that serum antihemagglutinin titers in convalescence were invariably higher when A-Baltimore-1003-57 virus was used as test antigen. It should also be noted that patient E.P., from whose throat washings we failed to isolate a virus, would have been undiagnosed if serologic studies had been done with A-Japan-305-57 virus alone.

Discussion. The term "P-Q-R variation" was coined by van der Veen and Mulder(3) to designate differences in avidity of antibody for influenza viruses of the same antigenic type. Viruses that are poorly inhibited by homologous and heterologous antiserum are said to be in the Q phase, as opposed to P phase viruses which are inhibited to high titer by homologous but not by heterologous antiserum. A virus in the R phase is inhibited to high titer by antiserum prepared from P, Q or R strains. Therefore, HI antibody determinations performed with Q phase virus as antigen will give fortuitously low titers. Isaacs and Andrewes(4,5) used these biological marker characteristics to great advantage in studying variants of A' influenza virus responsible for the epidemics of 1950-1951. In their studies Q phase viruses predominated in early outbreaks and were gradually replaced by P phase viruses, although both forms were sometimes present in a population at the same time. They also demonstrated that P-Q transformations could be reproduced in the laboratory by infecting animals under conditions which simulate those in human epidemics. P phase variants of A-Sweden-3-50 (Q) virus emerged after passage in the lungs of non-immune mice. Conversely, P phase virus was gradually converted to a Q form by passage in chicken embryos in the presence of subinhibitory doses of homologous antiserum. These authors also demonstrated that the P-Q-R variation is not an all-or-none phenomenon, but that a spectrum of strains exists with intermediate susceptibility to antibody.

The present studies demonstrate that in Oct. 1957 Baltimore strains of Asian influenza virus were isolated in the R phase in contrast to the Q phase A-Japan-305-57 virus isolated 7 months previously. Chickens immunized with the Japanese strain invariably developed higher HI and neutralizing antibody titers when serum was tested with Baltimore viruses rather than with the homologous strain. In fact, intradermal vaccination of human subjects with A-Japan-305-57 virus would have been considered totally inadequate if HI antibody response had been measured with the homologous (Q) strain alone. Serologic studies of patients convalescent from influenza were far easier to interpret when carried out with antibody-sensitive Baltimore (R) viruses. Nevertheless, the Baltimore strains of virus were indistinguishable antigenically from the prototype Asian strain as determined by cross hemagglutination-inhibition tests with chicken antisera. Although it has been suggested(3,4,5) that P and R phase viruses are more effective immunizing agents, similar HI titers were produced in chickens immunized with Asian strains in either the Q or R phase.

Summary. Asian influenza A viruses isolated during the Baltimore epidemic in Oct. 1957 were in the R phase of the P-Q-R variation although indistinguishable antigenically

from the Q phase prototype virus A-Japan-305-57.

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Received April 18, 1958. P.S.E.B.M., 1958, v98.

Pineal and Hypophyseal Phosphate Uptake After Castration and Administration of Sex Hormones.* (24044)

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Physiological roles attributed to the pineal organ in terrestrial vertebrates have usually involved gonads and hypophysis, but relationships to the control of blood pressure and general body activity have been suggested also (1-10). Unfortunately, many results are contradictory and are not supported by quantitative or statistical documentation. The suggestive and significant studies of Borell and Orström(11-15) on pineal phosphate uptake or turnover merit reexamination. Their finding that rat pineal has a turnover rate 3 to 4 times that of "adenohypophysis" and much greater than that of various parts of brain has been confirmed by Reiss, Badrick and Halkerston(16). Although incorporation of P^{32}-labeled phosphate into pineal phospholipids and proteins as well as into carbohydrate phosphate esters occurred within 40 minutes of intraperitoneal injection(12), the major portion of P32 uptake can justifiably still be considered as an indicator of metabolic rate, as it is generally in other organs. Among interesting results obtained by Borell and Orström (11-13) with female rats are: (1) increase in phosphate turnover in hypophysis and tuber cinereum and decrease in ovary and uterus after pinealectomy (operated at 50-100 g; sacrificed 5 weeks to 9 months later), and (2) increase in hypophyseal and pineal phosphate turnover 6 weeks after castration (6 ♂'s and 6 ♀'s). Apparently, tissue samples were combined to facilitate P determinations and no standard errors are provided. They postulated that pineal activates ovaries and conversely is inhibited by them(13). This conclusion contradicts the view held by other workers that the pineal is primarily antigonadal or antihypophysis(10).

Materials and methods. Female rats of Long-Evans and 2 mixed strains were used. In most experiments, litter-mate experimental and control animals were used in pairs and handled in identical manners. All animals received Diablo Double Check Labration pellets and tap water *ad lib.* Animal room temperature was 75-80° F. and artificial illumination was provided in single daily photo-periods 12-14 hours. Bilaterally ovariectomized rats and their sham-operated controls were maintained 3 weeks to 2 months (Table I) before autopsy. Daily subcutaneous injections of 17β-estradiol (Schering) were administered in 0.2 ml sesame oil during 4 days preceding autopsy. Injections of progesterone ("Proluton," Schering) were administered similarly in daily doses of 0.1 ml (5 mg) during 3 days preceding autopsy. Control animals were injected similarly with equivalent volumes of

* This investigation was supported by research grants from National Science Foundation, Nat. Inst. of Health, P.H.S., and Cancer Research Funds of Univ. of California. We are grateful to Dr. Preston L. Perlman of Schering Corp., for hormones, to Depts. of Genetics and Psychology for some animals used, to Drs. Morgan Harris, H. A. Bern, and K. B. DeOme for facilities and to Jean Grant and Tom Fashinell for technical aid.