supply for the mediocortical amygdaloid field or whether some other arrangement exists. Application of the new, Fink-Heimer technique to appropriate species and careful observation of the distribution of terminal degeneration in and about the amygdaloid complex should readily resolve this question.

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8. Before the report of L. Heimer [*Ann. N.Y. Acad. Sci.* **167**, 129 (1969)] on the central connections of the accessory olfactory bulb in the tegu lizard, the only well-documented report known to the authors on this subject in in lower forms was the Golgi study of C. J. Herrick in the frog [C. J. Herrick, *J. Comp. Neurol.* **33**, 213 (1921)]. In the tegu, the accessory olfactory bulb projects to the nucleus sphericus of the posterior telencephalon and in the frog it projects to the so-called amygdala. The latter finding has been confirmed in limited observations in our own laboratory in material studied by the Fink-Heimer method (F. Scalia, in preparation). Neither the reptilian nucleus sphericus nor the amygdala of the frog have clearly identifiable counterparts in the mammalian brain as studied by descriptive morphological analysis. Our study on the projections of the accessory olfactory bulb of the rabbit may provide a valid bridge, however, between the mammalian and inframammalian morphology.
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12. In considering our data, we have argued that even in those cases of lesions of the accessory olfactory bulb in which no apparent damage was done to the neighboring efferent fibers of the main olfactory bulb, it ought to be assumed nevertheless that those fibers were somehow injured until evidence to the contrary is obtained. If that assumption were warranted and if the main olfactory bulb does contribute to the synapses in the mediocortical amygdaloid nuclei, then there ought to have been some indication of terminal degeneration in that part of the amygdala whenever a lesion of the olfactory bulb resulted in degeneration of fibers passing close enough to the accessory olfactory bulb (two cases, 4 days' survival) to have been injured by a lesion of the accessory olfactory bulb. As already stated, however, in no case in which the accessory olfactory bulb was not damaged was degeneration observed in the mediocortical amygdaloid zone illustrated in Figs. 2 and 3. Therefore, the assumption, even if warranted, is of little significance.
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11 June 1970

Hemagglutination Assay for Antigen and Antibody Associated with Viral Hepatitis

Abstract. *Hemagglutination assays are described for measuring hepatitis-associated Australia antigen and antibody. Red cells coated with isolated antigen, with chromic chloride as a coupling agent, are used for detection of antibodies. Detection of the antigen in serums depends on inhibition of hemagglutination. The test has the sensitivity and rapidity of the best tests available, is simpler to perform, and lends itself to large-scale screening of blood donors.*

The finding of a specific antigen, popularly known as Australia antigen (*1*), in the serum of many patients with viral hepatitis has led to evaluation of a variety of techniques to provide a simple, rapid, and sensitive test for diagnosing hepatitis and detecting blood donors who may transmit the disease (*2–6*). At present complement fixation (CF) procedures appear to be most sensitive and rapid, immunodiffusion (ID) techniques are simplest but least sensitive and slowest (*7*), and electrophoretic modifications of precipitin techniques are as rapid as CF but are relatively insensitive (*5*). We now describe a hemagglutination (HA) technique for measuring hepatitis-associated antigen (HAA), which is as sensitive and rapid as the CF technique for detecting HAA and is easy to perform with reagents that can be standardized for use over long periods. The technique is more sensitive than CF and at least as sensitive as radioimmunoassay (*6*) for detecting antibody to HAA. In the HA test the agglutinogen consists of inert indicator red cells coated with isolated HAA with chromic chloride as a coupling agent (*8*). The agglutinator is antibody to HAA, and detection of HAA in serums depends on inhibition of agglutination (*9*).

Plasma samples from asymptomatic carriers of HAA were used to isolate purified antigen. Isolation was carried out by combined rate and isopycnic zonal centrifugation (*10*) with cesium chloride density gradients (*11*). The purified preparations were dialyzed extensively against 0.85 percent sodium chloride (saline) at 4°C. The Kjeldahl nitrogen content of the purified HAA used was 27 μg/ml, and the optical density at 280 nm wavelength was 0.445. This antigen preparation gave a single band in agarose at a maximum dilution of 1:2 with antiserum to HAA, and when concentrated tenfold gave no precipitin band with rabbit antiserum against whole human plasma.

Indicator cells were prepared from group O human blood collected in ethylenediaminetetraacetate. Cells were stored at 4°C for no more than a week prior to being coated with HAA. Red cells were washed four times with saline and used as a 40 percent suspension in saline. To coat the cells, 0.025 ml of cell suspension was mixed with 0.075 ml of purified HAA, and 0.025 ml of 1.25 m*M* chromic chloride was added. The mixture was agitated gently in a glass tube (10 by 75 mm) at room temperature for 5 minutes. Cells were then washed four times with saline and used as a 0.2 percent suspension in phosphate-buffered saline, *p*H 7.3, containing 0.5 percent bovine serum albumin (BSA), 0.0025 percent polyvinyl pyrrolidone (PVP), and a 1 : 20,000 dilution of Tween 80 (*12*). These conditions of coating were found to be optimum by a "checkerboard" test with different dilutions of HAA and chromic chloride (*9*).

Hemagglutination reactions for detection and titration of antibodies were carried out in V-shaped microtiter plates (*13*). The specificity of antibody to HAA was established by hemagglutination inhibition (HAI) with purified HAA and known HAA-positive serums and failure to inhibit agglutination with known HAA-negative serums (*12*).

In testing for HAA, ten times the minimum amount of antibody that gave a positive agglutination was mixed with an equal volume of various dilutions of test serum, and then HAA-coated cells were added. The antiserum to HAA used had a titer of 10,000 in HA, 480 by CF, and 8 by ID. Serum containing HAA used as a known positive control was from a patient with serum hepatitis and had a titer of 1280 by HAI, 1000 by CF, and 8 by ID. Serum from a normal individual that had contained no antigen by these tests was used as a negative control.

Table 1. Antibody assay on 72 serums by HA, CF, and ID. HA titers are reciprocal dilutions of serum showing agglutination.

No. of serums	HA titers	Number of serums positive in	
		CF	ID
46	0	0	0
2	4	0	0
6	8–128	0	0
10	256–2,560	9	0
6	2,560–40,000	6	6

A total of 72 serums was tested for antibodies by CF, ID, and HA (Table 1). The two serums with titers lower than 8 could not be tested for specificity of antibody by HAI (*12*). Antibodies with HA titers of 8 or higher were found to be specific for HAA by the HAI assay. The sensitivity of HA appeared to be at least 2000-fold greater than that of ID and 100-fold greater than that of CF. All antibodies detected by CF or ID were detected by HA.

To test for HAA in serum samples, 0.025 ml of test serum at 2-, 4-, 8-, and 16-fold dilutions were mixed with 0.025 ml of antibody to HAA containing 10 agglutinating units as defined above, and then 0.025 ml of cells coated with HAA was added. As a control, a 1:2 dilution of each serum was tested with HAA-coated cells in the absence of antibody to HAA. A rare agglutination in the control system (2 of 523 serums tested) was checked with the noncoated cells to rule out red cell isoantibodies. The one positive sample in this test system proved to contain specific antibody to HAA and the second one was too weak to determine specificity. In all, 523 serum samples obtained from patients during the incubation period, acute phase, and convalescent phase of viral hepatitis were tested by HAI, and the results are compared in Table 2 with those of CF and ID performed previously (*7*). The test for HAI was read as follows: partial inhibition of agglutination in 1 : 2 dilution was considered negative, definite inhibition of agglutination in 1 : 2 dilution was a weak but definitely positive test for the presence of HAA, inhibition of agglutination in 1:4 and higher dilutions was a more strongly positive test for HAA. As shown in Table 2, the sensitivity of the CF and HAI is similar, whereas the ID test is much less sensitive. Of the 142 positive results with CF, 29 specimens were strongly anticomplementary, indicating the presence of large amounts of antigen-antibody complexes (*3*). Five samples of another patient that were negative in CF were positive in HAI at a dilution of 1:2.

Table 2. Detection of HAA antigen in a panel of serums tested previously by CF and ID (*7*).

Assay	Results		
	Negative	Weak positive	Positive
ID	463		60
CF	326	55	142
HAI	321	60	142

Of the various techniques now in use, the plain immunodiffusion test, while easiest to perform, is least sensitive and requires 1 or more days for completion. The use of electrophoretic techniques, either with agar-gel or cellulose acetate membranes, permits tests to be completed within 2 hours; the gel electrophoretic procedures increase the sensitivity of immunodiffusion approximately fivefold (*4*), while the cellulose acetate technique does not increase sensitivity (*5*). The CF tests can be completed within 2 hours and are approximately 100-fold more sensitive than ID techniques for detecting the antigen and about 20-fold more sensitive for detecting the antibody. Semiquantitative or qualitative CF techniques with microtiter plates can be performed in a routine manner and also can be automated. For smaller laboratories not equipped to standardize reagents for the CF tests, gels incorporating sensitized cells permit the CF test to be carried out as easily as the ID test (*14*). One unique advantage of the CF assay is its ability to detect circulating antigen-antibody complexes by their anticomplementary properties. The HAI and CF techniques have similar sensitivity for detecting HAA. The HAI can be applied with reagents that can be stored for at least a week and potentially longer, if the cells are fixed in glutaraldehyde (*15*). The test also lends itself to automation.

A recently developed radioimmunoassay (*6*) appears to have about the same sensitivity as CF and HAI for detecting HAA and sensitivity similar to that of HAI for detecting antibody. Since the radioimmunoassay is very complex, requires large amounts of specialized equipment, and takes about a week to perform, it appears that the HAI test is superior. Of all the techniques to date, the HA and HAI tests compare favorably with respect to rapidity, sensitivity, and simplicity, and they should be as practical as any currently available laboratory procedure for large-scale screening of blood donors and for rapid diagnostic tests for hepatitis.

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16. Supported by PHS research grant Am-14514-01. We thank C. Knepp and C. Coleman for technical assistance.

28 July 1970