tradiction between social structure and demography in the central institution of the Galla of Ethiopia, *Gada*, generated change and how the combined social-demographic system underwent orderly transformation for several centuries. Legesse, himself an Ethiopian, sees clearly how Western anthropology is in many ways a "construct" of intellectuals observing the Third World from the privileged Western corner of world culture.

3. V. Turner, *The Drums of Affliction* (Clarendon, Oxford, 1968), pp. 198–268.
4. C. Geertz, *The Interpretation of Cultures* (Basic Books, New York, 1973), p. 448.

12 October 1973

Nonspecificity of Hepatitis B Antigen Detected with Iodine-125–Labeled Antibody

The state of the art in hepatitis B antigen (HBAg) testing was reviewed a year ago (*1*). Counterelectrophoresis (CEP), the technique routinely employed by blood banks for the detection of carriers of HBAg, was recognized to be insufficiently sensitive. The most sensitive procedures under investigation involved radioimmunoassay (RIA). Although RIA procedure by the double antibody technique described by Hollinger (*2*) is both sensitive and specific for the detection of HBAg (*1*), a commercially produced solid phase RIA procedure, based on the "sandwich" principle, in which ^{125}I-labeled antibody specific for HBAg is used (*3*) has been licensed for sale by the Food and Drug Administration (*4*) and has been placed in routine use by many blood banks.

The relative sensitivity of this licensed RIA technique is unquestioned, but direct proof of *specificity* could not be shown when the reactive samples were negative by other techniques. The report by Ling *et al.* (*5*) appeared to validate the specificity of the RIA-reactive samples by demonstrating that they could be subtyped into ad and ay as had been established by LeBouvier (*6*). However, the implication of that report that all RIA-positive reactions can be considered specific for HBAg is now contradicted by evidence obtained in this and other laboratories (*7*). The data presented here illustrate the problems encountered with the RIA test and include evidence that most of the RIA-positive, CEP-negative results are false positives, not related to the presence of HBAg.

A total of 9249 serums of consecutive voluntary blood donors were screened for HBAg by CEP and subsequent RIA testing according to the directions of the manufacturer of the kits (*8*). Table 1 presents the results obtained with the 9249 consecutive blood donor samples. Each of the 12 samples positive by CEP were also positive by RIA. No samples have been found to be CEP positive and RIA negative. Of the 121 RIA-positive, CEP-negative samples only 30 were consistently positive at 2.1 times the control, and 1 sample was consistently positive at 1.5 times the control. In large part this poor reproducibility was caused by variable sensitivity of different lots of reagents, as judged by two RIA-positive serums weakly reactive at 2.0 times the control and by a reference standard whose titer was checked with different lots. With the 306 serums where the initial reaction was 1.5 to 2.1 times that in the control range, reproducibility of results was so poor (2 out of 36; 6 percent) that no further studies of these serums were carried out. The manufacturer of the RIA kits became aware of the false-positive problem discussed below (*9*). The following studies were done with the new materials supplied by the manufacturer.

The reproducible RIA-positive samples from our original studies were combined with reproducible RIA-positive samples from other laboratories (*10*) to provide a total of 52 samples. The specificity of the RIA-positive reactions was investigated by an inhibition technique (*7*). Inhibition of the reaction by human antiserum to HBAg (titer 1 : 2000 by hemagglutination) was considered to validate the specificity of the reaction for HBAg. Inhibition of the reaction by normal guinea pig serum was taken as evidence that the positive results were caused by a reaction between the test serum and guinea pig proteins (the antiserum to HBAg used in the RIA test are of guinea pig origin). In the control incubations we used normal saline and human serum in which HBAg and antiserum to HBAg could not be detected. The technique was as follows: 0.1 ml of test serum was incubated with 0.1 ml of inhibiting material overnight at 4°C. The entire mixture of 0.2 ml was then transferred to the RIA test tube, and the test was then carried out as usual.

Table 1. Results of consecutive testing of 9249 blood donors and repeat test to confirm the first positive results.

Serums	First test	Repeat test +
Total tested	9249	
CEP positive	12	12
2.1 × negative control	133	43
1.5 × negative control	306	2*

* Thirty-six consecutive samples were repeated.

The results are shown in Table 2. The test serums were divided into four groups; within each group the reactions were closely similar. Only 4 of the 52 samples (7.7 percent) were inhibited by antiserum to HBAg (group 1). In contrast, 32 samples (61.5 percent) were completely inhibited by normal guinea pig serum (group 2). An additional three samples (group 3) were significantly inhibited by guinea pig serum (more than 5 standard deviations), but the counts per minute were still slightly higher than the 1.5 times control considered to be true reactive results. Combining groups 2 and 3, there were 35 samples (67.3 percent) whose reactions may be attributed to antibody to guinea pig protein. Group 4 consisted of 13 samples (25 percent) whose reactions were very close to the 1.5 times control counts. Addition of antiserum to HBAg or guinea pig serum either had no effect or produced minimal and nonreproducible inhibition. No conclusions can be drawn as to the cause of the reactions in this group.

The conclusion that only 7.7 percent of the RIA-positive, CEP-negative results were true positives is in conflict with that of Ling *et al.* (*5*). However, in their investigation only 24 of the 85 serums studied were CEP negative, and most of these specimens had count rates (4.2 to 7.2 times the normal control) much higher than those we observed in unselected RIA-positive, CEP-negative samples from normal blood donors (generally less than 3.0 times the control). The specimens reported by Ling *et al.* reflect a distribution of ad and ay subtypes normally not found in unselected donor populations, and in their report there is no indication of how they selected the 85 samples.

Recognition that RIA-positive, CEP-negative reactions in a volunteer blood donor population are almost all false positives has important practical implications. Unnecessary permanent rejection of a healthy donor impairs the ability to provide blood from volunteer donors. More important, the donor

Table 2. Specificity of RIA+/CEP− serum samples tested by neutralization of serologic activity by human antiserum to HBAg and normal guinea pig serum. Controls consisted of addition of normal human serum and saline in separate tests. The numbers in parentheses indicate the range of the ratios in each category.

Group	Serums (No.)	Ratios of the mean number of counts per minute of sample to negative control after neutralization with			
		Human antiserum to HBAg	Normal guinea pig serum	Normal human serum	Saline
1	4	1.2 (1.0–1.3)	9.0 (2.6–14.0)	10.1 (2.4–16.0)	10.1 (2.3–16.4)
2	32	2.6 (1.5–26.4)	1.2 (1.0–1.4)	2.9 (1.5–26.4)	2.9 (1.5–28.5)
3	3	2.9 (2.2–3.6)	1.6 (1.5–1.8)	2.4 (2.1–2.8)	2.6 (2.1–3.1)
4	13	1.5 (1.2–1.8)	1.4 (1.1–1.6)	1.5 (1.1–2.1)	1.3 (1.0–2.0)

falsely labeled as a hepatitis carrier is subjected unnecessarily to anxiety about his health, fear of contagion to others, potential loss of employability in certain occupations, the expense of investigation of liver function, and possibly the risk of a liver biopsy.

Additional disadvantages of the existing licensed RIA tests include a significant increase in the cost of processing blood for transfusion, varying sensitivity of different lots of reagents, and a 20- to 24-hour delay between the beginning of testing and the availability of the results. Current demands for fresh blood components (especially platelets) dictate the administration of at least a fraction of 20 to 30 percent of collected units before the RIA test results are available.

The RIA test appears to be unsuitable for routine blood donor screening at the present time. Further research is required to improve its speed and specificity, and to establish that it detects CEP-negative donors who have a significantly greater chance of transmitting hepatitis than donors whose serum is nonreactive. Routine RIA testing on a national basis will increase the cost of processing the 7 to 8 million units of blood collected annually by at least $3 per unit. The detection of additional dangerous carriers should be frequent enough to justify the large additional investment of patient money for health protection, taking into consideration the rising costs of health care and the possibility that the money could be better spent for protection of the nation's health in other ways. Other sensitive procedures such as hemagglutination inhibition or reverse passive agglutination may prove to be preferable to RIA with further improvements. Meanwhile, it is recommended that a blood donor whose serum is reactive in the RIA test be considered a carrier of HBAg only if the result can be confirmed by a more specific method, or if the RIA result can be specifically neutralized by antiserum to HBAg.

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7. A. M. Prince, B. Brotman, D. Jass, H. Ikram, *Lancet* **1973-I**, 1346 (1973); H. F. Polesky, and C. Olson, in *Presidential Memorandum* (American Association of Blood Banks, 18 April 1973).
8. According to the directions of the manufacturer, which are approved by the Food and Drug Administration, samples are to be considered nonreactive in the RIA test unless their counts per minute are more than 2.1 times that of the mean negative control sample. Samples showing more counts per minute than 2.1 times that of the negative control are to be repeated, and may be considered reactive if the counts per minute are more than 1.5 times the control.
9. Mailgram—"AUSRIA test kits shipped to you February 6, 1973, through March 22, 1973, are reported to be giving an increased number of repeatable, non-specific false positives due in part to cross reactivity of human serum and guinea pig protein. Donors testing positive with these lots may be negative and should be held for retesting with new materials which will be supplied to you as soon as possible. A letter giving details will follow immediately. Radio-Pharmaceutical Products Division Abbott Laboratories."
10. Additional RIA-positive, CEP-negative samples were provided by Delores McGuire of the Community Blood Bank, Dayton, Ohio, and Granville Harrison of Denver, Colorado.
11. We thank Drs. Robert K. Ockner and Stephen N. Cohen for their comments. Supported by the National Blood Resource Branch of the National Heart and Lung Institute under contract 71-2355.

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In that the commercial RIA test for hepatitis B antigen (Ausria) is an outcome of our research (*1*), we feel it necessary to point out that Vyas, Adelberg, and Perkins (*2*) apparently do not recognize that our report was principally directed to the use of ^{125}I-labeled antibody to subtype human serums containing the hepatitis B antigen (HBAg).

Our report (*1*) dealt exclusively with the methodology of the use of ^{125}I-antibody to HBAg for classifying serums into the d and y subspecificities of LeBouvier (*3*). The facts we reported were:

1) Radiolabeled type-specific antibody was used to classify HBAg-positive serums into d and y subsets with perfect correlation with agar gel diffusion (AGD) results.

2) The technique was also useful in classifying low-titered AGD-negative serums into subspecificities.

3) Of 85 serums obtained from commercial donors in late 1971 and early 1972, 81 were subtyped into d and y specificities. Four serums showed variations from these specificities.

4) Classification into d and y subspecificities with radiolabeled antibody was a convenient way to validate serums positive only by radioimmunoassay.

We have information on the frequency of d and y subtypes, on the number of neutralizable and subtypable positives in various blood donor populations, and on the specificity of our reagents. In that this information was not presented (*1*), we now report one of our studies that serves as a useful comparison with the Vyas study.

Vyas *et al.* analyzed 9249 serums and found 43 (*2*, text: 12 + 30 + 1) or 45 (*2*, table 1: 43 + 2) positive by commercial ^{125}I-labeled HBAg antibody available during the first half of 1973. Twelve of these serums were positive by counterelectrophoresis (CEP), presumably by commercial reagents also.

We do not know whether the CEP analyses were made retrospectively or were found in an original screening by CEP. The 31 or 33 CEP-negative specimens were then validated by neutralizing with an unspecified human antiserum in a procedure requiring a twofold dilution of the test sample. The same amount of antiserum was used, whether the specimen contained several milligrams of HBAg per milliliter or a nanogram per milliliter. There is no information that the CEP-positive specimens were neutralized by this procedure. It is not possible to assess

Table 1. Confirmation and subtyping of HBAg positives from 5344 consecutive blood plasmas; NHS, normal human serum; HAb, hepatitis antibodies.

Group	Samples (No.)	Ratios of the mean number of counts per minute in the sample to the mean number of counts per minute in the negative control					Subtypes
		Detection	Confirmation		Subtyping		
			10 percent NHS	10 percent HAb	Anti-d	Anti-y	
			CEP positive: 22				
1	14 (34.1%)	23.5 (12.4–58.0)	23.0 (10.0–63.0)	3.4 (1.1–7.2)	192.6 (11.1–262.2)	1.5 (0.8–2.3)	ad
2	8 (19.5%)	14.2 (10.6–16.7)	11.8 (5.7–18.9)	2.1 (0.9–5.3)	2.1 (1.2–3.1)	73.4 (47.9–110.6)	ay
			CEP negative: 19				
3	5 (12.2%)	20.8 (14.9–23.4)	21.0 (13.4–27.9)	1.7 (1.2–2.3)	60.9 (20.6–153.3)	1.0 (0.6–1.4)	ad
4	5 (12.2%)	13.3 (5.7–22.5)	13.7 (4.0–23.1)	1.7 (0.9–3.7)	1.8 (1.0–3.6)	26.5* (1.9–77.9)	ay
5	9 (22.0%)	2.7 (2.1–4.0)	2.8 (2.1–4.5)	2.9 (2.2–4.2)	1.3 (0.9–1.9)	1.7† (0.8–3.2)	Equivocal

* Sample 2478 in group 4 had a ratio of 20.8 in detection, and was confirmed HBAg positive by neutralization, but the ratio was only 1.4 with ^{125}I-labeled anti-d and 1.9 with ^{125}I-labeled anti-y; this sample may contain a subtype predominantly. † Sample 38 in group 5 was not confirmable by neutralization test; yet it appeared to be apparent ay subtype (the ratio was 1.2 for ^{125}I-labeled anti-d and 3.2 for ^{125}I-labeled anti-y).

how many of the serums were neutralized, because they were combined with 21 or 22 nondocumented, selected serums from Dayton, Ohio, and Denver, Colorado. One must wonder why a perfectly good study with 9249 serums from consecutive donors in California was comprised by adding into the study another 21 or 22 positive serums selected at two other places. The neutralization results of the combined total of 52 specimens are presented by Vyas *et al.* in their table 2. Thirteen of the samples could not be resolved one way or the other by their neutralization procedure. Assuming that these may be negative, we believe that three conclusions can be made from the studies:

1) About 15 or 16 confirmed positives were found in 9244 consecutive donors using ^{125}I-labeled antibody.

2) The RIA positives represented about 30 percent more than could be found by CEP under the best conditions.

3) In this population there were about three nonneutralizable positives per 1000 donors with the reagents obtained prior to June 1973.

One of our recent studies appears germane to the Vyas report. It involved the analysis of the serums of 5344 consecutive commercial blood donors from the West Coast. All the samples were tested by CEP and by ^{125}I-labeled guinea pig antibody (*4*). The ^{125}I-antibody was a mixture of antibodies to a, d, and y determinants of HBAg, and was mixed in a solution of bovine serum (30 percent), normal guinea pig serum (20 percent), normal human serum (0.2 percent)—the present commercial reagents. The CEP analyses were made retrospectively, with the operator knowing the RIA results. Samples repeatedly showing count rates greater than 2.1 times the negative control mean were subjected to confirmation with pooled human antiserum with a rheophoresis titer of 32 against a battery of panels of antigens. Each positive serum was also subjected to confirmation by subtyping with ^{125}I-type specific antibodies (*1*). For the neutralization test, we used the human antiserum at a level of 10 percent, mixed with the labeled antibody in the second step of the test procedure (*3*). This minimizes the disadvantages of a procedure, as reported by Vyas *et al.*, which causes a twofold dilution of the sample and also permits insufficient neutralization of samples with high HBAg titers or HBAg-antibody complexes. In our procedure, a sample was considered confirmed when the count rate was reduced by more than 50 percent with human antiserum as compared to a normal human serum control.

The results of this study are summarized in Table 1. Of the 5344 consecutive donor units, 22 were positive by CEP and with ^{125}I-labeled antibody (group 1 and 2), and 19 additional units were positive with ^{125}I-labeled antibody only. All of the 22 CEP- and RIA-positive units were confirmed by neutralization and by subtyping, while only 10 units (groups 3 and 4) of the 19 CEP-negative units were confirmable. In separate experiments, we also tested all of these 41 RIA-positive samples, using heterologous systems; for example, ^{125}I-labeled human antibody to HBAg and tubes coated with guinea pig antibody to HBAg, or vice versa. In both cases only the 32 confirmable units were positive and the 9 nonconfirmable units (group 5) were negative (data not shown). Thus, it appeared that the 9 nonconfirmable positives resulted from the use of guinea pig antibody for both the solid phase and ^{125}I-labeled antibody solution.

All but one of the confirmed HBAg positives were subtyped into d (groups 1 and 3) and y (groups 2 and 4) subtypes, and all but one of the nonconfirmable positives (group 5) were not distinguishable as to d or y subtypes (Table 1). These two exceptions (footnotes to Table 1) should be of particular interest to anyone choosing a single technique for confirming HBAg-positive serums. In this study there was one serum that could be neutralized with human antiserum but could not be subtyped as d or y. Also there was one serum that was not neutralizable with our antiserum, but followed the pattern of y subtype. Thus, each test is very useful, but neither test may be unfailingly reliable.

We conclude from our study of 5344 consecutive commercial donor serums the following:

1) Thirty-two serums were found positive with ^{125}I-labeled guinea pig antibody and confirmed by neutralization and subtyping.

2) The 32 RIA positives represented about 45 percent more than could be found by CEP under the best laboratory conditions.

3) In this population there were about two nonconfirmable positives per 1000 donors.

It appears to us that these three factors represent the most efficient way of assessing and comparing various

testing procedures. If the number of cross reactants is reasonably constant across all populations and if the number of specific reactants varies widely with the population, then the *percentage* of confirmable positives compared to nonconfirmable positives will vary widely.

In our study we found 19 ad-type serums and 13 ay-type serums, very similar to our previous study where the distribution was 47 ad and 34 ay. Vyas *et al.* state that this "reflects a distribution of ad and ay subtypes normally not found in unselected donor population." We believe our findings are correct, and suspect that they are normal.

The cost and value of testing for HBAg have been subjected to much discussion. Hopefully, the value scale does not depend on whether one is a recipient of a blood transfusion, a blood donor, or a blood bank scientist. The primary purpose in hepatitis B screening of blood is to protect the recipient. It is our opinion, based on these and other data, that at the present time RIA does this better than CEP. Measures must be taken to protect the donor also, but not at a risk to the recipient. Until all commercial hepatitis tests are free of false positives, confirmatory testing, such as neutralization, will be necessary and good judgment is essential.

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25 October 1973

Artifacts of Early Man in the New World

Vance Haynes, in his article "The Calico site: Artifacts or geofacts?" (*1*), makes the important point that the maximum age of the site is still very much in doubt, although the minimum age was established by carbon-14 tests at the University of California at Los Angeles as "greater than 50,000 years" (*2*, p. 15). He is also quite right in insisting that the products of the natural processes to which the chert fragments were subjected require more study than that presented so far by Oakley (*3*) and Jelinek *et al.* (*4*). However, I believe he is neglecting the factor of logically patterned flaking also pointed out by Oakley (*5*) in distinguishing artifacts from geofacts.

For example, what would be the probability of natural processes producing a series of 8 to 15 flakes detached alternately from opposite sides of one edge of a piece of chert, each flake of very nearly the same size—that is, produced by the same amount of force applied alternately in opposite directions? Flakes of varying sizes and random alternation would be expected, but the Calico collections have many specimens of cherts with a long series of edge flake scars of very similar size and precise alternation. I personally witnessed the removal of one such piece from section J-13, on the 306- to 309-inch level below datum (1 inch = 2.54 cm), last winter.

Natural processes in the alluvial fan might well produce a few cherts shaped like primitive tools, but the number of tool-shaped cherts of set and regular Paleolithic patterns found in the really very small area excavated so far would appear to be overwhelmingly beyond the probability of accidental fabrication. Over 600 "tools," plus another 1500 technically diagnostic flakes, were selected by L. S. B. Leakey before his death.

One would expect that natural processes producing such pieces would form them indiscriminately from any quality of chert. The "artifacts" selected by Leakey as undeniably man-made have all been of excellent quality stone, although both good and poorer quality material is present in abundance. Such selectivity is characteristic of man—is it characteristic of nature?

In another aspect of patterning, it appears that Haynes is in error. The artifacts are found only in the lower Yermo formation, as he says, but they are not randomly distributed, either vertically or horizontally. There are definite concentrations at 2 and at 3 m below the juncture with the sands of the upper Yermo formation. They are also concentrated toward the northwest corner of master pit 1 and the southeast corner of master pit 2 (*2*, pp. 39, 40, and 42). The center of the site may well lie between the two pits.

When Haynes says, "No specimen from Calico is as obvious an artifact as, for example, a typical Chellean hand ax, a Levallois flake, or a Mousterian point" (*1*, p. 307), he is unfortunately expressing as fact what is only an opinion. Leakey disagreed with him here, as did François Bordes, the French expert in Paleolithic tool types and technologies, who was particularly interested in the diagnostic flakes. Agreeing with Leakey and Bordes are Pierre Biberson of the Institut de Paleontologie Humain, Paris, and Yves Coppens of the Musée de l'Homme, also in Paris. Because of differences in their training and viewpoint, it seems to be easier for those experienced in the prehistory of the Old World to accept the Calico chipped flints as man-made; however, the flints are now accepted as man-made by at least 75 percent of the professionals who have visited the site and inspected the collections (*6*).

Haynes is to be commended for bringing this controversial site to national attention. Now that the possibility of very early man in the New World has been broached, a number of people are actively searching for more evidence, believing they will find it. Let us hope that some of the new sites will be easier to date geologically.

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6. R. D. Simpson, personal communication.

23 August 1973

Wade's comment is an excellent example of the problems encountered in interpreting the Calico collections. Much depends upon the opinions of the observer, and different observers have different opinions, even in regard to what is considered fact.

In the stated case of "many specimens of cherts with a long series of edge flake scars of very similar size and precise alternation," the facts are (i) they are made of chert and (ii) they have a series of edge flake scars. The