Radiocarbon Dating and Palaeoserology of a Selected Burial Series from the Great Cave of Niah, Sarawak, Malaysia

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Introduction

In 1966, R. H. and S. T. Brooks received a National Science Foundation (NSF) Grant, number GS-1054, for a cooperative research project with the Sarawak Museum, Kuching, Malaysia, at the Great Cave of Niah, Sarawak, Malaysia. The Sarawak Museum provided transportation and some funding within Sarawak, and the project was implemented through the assistance of Museum personnel, especially Tom Harrisson, then Director of the Museum, Barbara Harrisson, and Lucas Chin. The intent of the project was to combine an archaeological and physical anthropological in situ analysis and recovery of specific burials. Data for the palaeoserology and radiocarbon dating analyses of selected burials from the series were obtained in the field for completion later in the laboratory. Two months were spent at the Great Cave of Niah in the summer of 1966, analyzing, recording, and excavating selected skeletons from the Niah Cave burial series. The previous year 71 burials had been removed by the Harrissons and sent to the Brookses in the United States to begin a preliminary laboratory analysis.

Bone from two burial areas was sent from the field to Isotopes, Inc. and also to Geochron Laboratories, Inc. as pilot samples to determine whether the human bone material could be radiocarbon dated. Samples of wood associated with one of the burials were included for comparison. The National Science Foundation was

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informed of the successful completion of the dating analysis and subsequently provided sufficient funds for the radiocarbon dating of 30 additional skeletons. Soil samples for the palaeoserology had been collected for some of the burials that had been excavated the previous year by the Harrissons. During the field project, soil samples from within the body cavity were collected for all burials in process of removal during the summer of 1966.

Although the bulk of the burials recovered over the several years of excavations at Niah Cave are now at the University of Nevada, Las Vegas, Physical Anthropology Laboratory, and have been analyzed in preparation for an anthropometric and morphological description of the series, not all of the more than 200 burials could be included in the palaeoserological and radiocarbon analyses owing to funding limitations. Radiocarbon dating was limited to the choice of the 3 burials dated during the 1966 field research; in 1973 the Rikagaku Kenkyusho (The Institute of Physical and Chemical Research), Wako-Shi, Saitama, Japan, conducted an analysis of 30 additional burials. These latter were selected to include key burials which Tom Harrisson felt should be dated to resolve archaeological problems and as many as possible of the burials which were being analyzed through palaeoserology. Factors in the selection of burials for dating were their location in the various parts of the Upper and Lower portions of Niah Cave or their burial positions.

The selection of burials for palaeoserological analysis began well prior to the 1966 field research. When the first 71 burials were sent to the United States for analysis, Rodger Heglar, now at the Anthropology Department, California State University, San Francisco, suggested that if soil samples from the exact burial locale could be obtained he would be willing to undertake the palaeoserological analysis. Thirty-four burials showed ABO blood grouping results during the bone blood research; 23 of these were removed during the 1966 field research and 25 were radiocarbon dated.

PALAEOSEROLOGY

Over several years, human bone samples and soil samples were sent from the Niah Cave, Sarawak, archaeological site to Rodger Heglar for palaeoserological testing. In total, 44 bone samples from burials and 193 soil samples were submitted. Of the latter, 63 samples were chosen as parallel soil controls for burials and to test the variety of soils apparent in the site.

Although bone blood-grouping is not new, it has had a frustrating history in research. Heglar has presented the history and the methods of this effort at both archaeological and biological anthropology annual national meetings, but the material to date has appeared in publication only in a journal not widely read by most anthropologists (Heglar 1972). Consequently, it is appropriate to explain here the testing procedures and logic of test results interpretations as promised in the preliminary report of Niah Cave palaeoserology (Brooks and Heglar 1972: 87).

Many archaeologists have been most critical of palaeoserology, their views having been influenced particularly by research in the late 1950s in which sources of contamination and irregularities in tests for ABO(H) antigenicity were stressed (Thieme and Otten 1957). During this same period and in subsequent years other workers were continuing to improve and control problems in ABO(H) bone typing reliability both in this country and in England (Gray 1958; Glemser 1963). In this

presentation, some minor procedural changes can be noted if comparisons are made with the methods of the former workers. In addition, the notion of parallel-testing bone and soil samples from the same archaeological burial as a "control" for bone contamination from the soil is suggested and utilized. The detection of ABO(H) blood group antigenicity in bone samples is assumed to reflect the secretor status of an individual, that is, the presence of an antigenicity in body fluids similar to that of the ABO system located on the red blood cells.

Testing Procedures and Interpretation

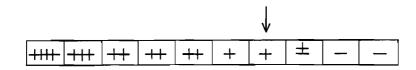
The methodology employed can best be summarized in the following step-by-step format:

- 1. Bone sample (thoracic or lumbar vertebra cancellous bone) is pulverized to talcum powder consistency. Soil samples were not prepared to a grain finer than received from the site.
- 2. Both bone powder and soil samples were weighed out into 0.5 gm amounts.
- 3. Three weighed amounts of each sample type were placed into labeled 10×75 mm test tubes marked as "Bone" A,B,H and "Soil" A,B,H. Each tube also indicated a laboratory number which was actually the Niah Cave burial number. This step was in preparation for the absorption tests.
- 4. To the appropriately labeled bone powder tube, 0.2 ml of titered antisera Anti-A₁, Anti-B, and Anti-H (titer strength of 1:64) was added. To the similarly labeled soil sample tubes a double amount of antisera was added to insure a proper recovery of at least 0.2 ml from the soil "mud" after centrifuging.
- 5. After mixing, the two sets of three tubes were refrigerated at 4-5°C for 12 hours and agitated at least every 1-2 hours.
- 6. All tubes were then centrifuged for one minute at 1500 rpm and the antisera decanted. This was labeled as "Run 1."
- 7. Next, the same amount of antisera in step 4 was added again to the same bone powder and soil sample tubes, and steps 5 and 6 were repeated.
- 8. The second decanted antisera were labeled as "Run 2."

The serological test utilized for the demonstration of A, B, and H antigenicity in these samples was the absorption test. Briefly, this method includes a 10-tube serial dilution, or titration, of each antiserum tested for red blood cell (RBC) agglutination against a set of known-type ABO RBC. Figure 1 presents the format and explanation of the titration process. Here it should be noted that the basic titration consists of a row of 10 tubes ($10 \times 75 \text{ mm size}$). Into tubes numbered 2–10 an equal amount of normal saline (0.1 ml) is added. Next, the same quantity (0.1 ml) of a specific antiserum is added to the appropriate tubes numbered 1 and 2. At this point a transfer takes place of 0.1 ml from tubes 2–10 with tube 10 being finally quantified by the removal of 0.1 ml.



EXAMPLE: RBC Agglutination = Antibody Titer (1:64)



Note 4+ to |+ = "strong" to "weak" Agglutination

Titer read at last |+ tube

Fig. 1 Recording format and titration interpretation.

Note that in Figure 1 dilution ratios are listed below each tube position. When each antiserum is transferred down the tube row in equal amounts it is in fact geometrically diluted by the saline in each tube. Specific blood group RBC are added to the row of tubes containing the specific antibody via the antisera. One drop of RBC per tube is allowed to stand at room temperature, after mixing, for 30 minutes. The tube rows were then centrifuged and read macroscopically for agglutination, that is, a "clumping" of RBC upon shaking under observation. Agglutination is read and recorded as a range from the strongest reaction (++++) to the weakest (+). Note in the example that the last "+" is read as the titer. Traditionally the tube following the last readable "+" is listed as doubtful and recorded as "+".

Figure 2, with the last "+" circled for easy recognition of its position, shows the differences in titer strengths in an example of the first and second "runs." Here a common occurrence is seen in the first run where the titer of several or all ABO(H) antisera has dropped from the expected "control" titer of 1:64 at the seventh tube. There are a number of reasons for this nonspecific effect that have to do with contaminating chemistry. In the second run, "antigen specificity" can be argued if the same sample still shows a drop of 3 titer tubes or more for 1 or more of the antisera. In the Figure 2 example, the second run for the bone powder sample shows an antigenicity for A₁ and H. Heglar prefers the 3 tube titer drop as a criterion for blood group determination in that it includes a possible 1 tube drop that can often be accounted for from titration or technician error. This same example also shows a second run of the soil sample that would not indicate antigenicity by the foregoing criteria. The A₁ antigenicity, and in this case the H as well, of the bone sample probably did not chemically come from the soil of the burial.

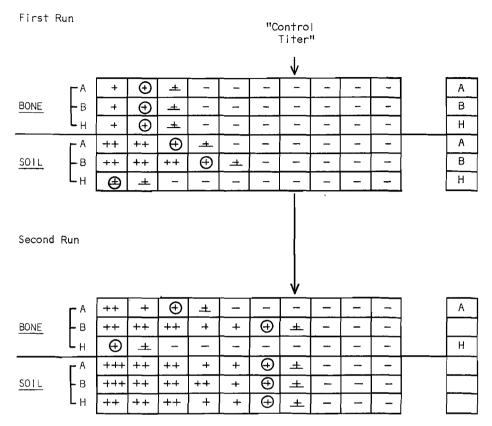


Fig. 2 Typical bone/soil parallel tests, Niah Cave, Borneo.

It is the serological logic described above that was operative in judging the bone blood-grouping data presented here for the Niah Cave burials. Only those samples that could be reasonably argued as containing specific antigenicity *not* influenced by their representative burial soil samples were considered as bone blood-grouped in the ABO system. On the laboratory record and titration forms, the occurrence of A_1 and H = blood group A_1 ; B and H = B; A, B, and $H = A_1B$; and only H = O.

Palaeoserology Results and Discussion

Table 1 presents the Niah Cave burial sample tests with their soil sample results plus some archaeological information. A total of 42 parallel bone to soil tests were run with 34 (81 percent) showing ABO blood grouping results under the logic described earlier. The 34 "typed" burials have a distribution as follows:

TYPE	NUMBER	%
O	20	58.83
A_1	11	32.35
B	2	5.88
A_1B	1	2.94

The soils, with one exception (no. 62A), indicated only H specificity. Of the 65 soil samples tested, 44 (68%) did not demonstrate any contributing A_1 , B, or H antigenicity that would alter the bone chemistry or test results.

The archaeological information supplied in Table 1 suggests a correlation between the extended burial position and the Upper Cave area of the site. The flexed burials, though few, were mainly in the Lower Cave region. As far as any temporal placement of burials and bone blood-grouping is concerned, the Early burials were represented by blood groups O, A_1 , and B. This time slot at the oldest is more than 11,000 years B.P., which is not particularly ancient for human populations in this part of the world.

TABLE 1. Archaeological and Serological Data for Niah Cave Bone and Soil Samples

	BURIAL DATA				BONE		SOIL			
BURIAL NUMBER				A_1	В	Н	$\overline{A_1}$	В	Н	
3	Ex	UC	L	X		X	_		X	
10	$\mathbf{E}\mathbf{x}$	UC	L	X	_	X			\mathbf{X}	
30	\mathbf{Fr}	LC	L			X				
36	Ex	UC	L			X			_	
49	Fr	UC		X	_	\mathbf{X}			\mathbf{X}	
50	$\mathbf{E}\mathbf{x}$	UC	L			\mathbf{X}		_		
51B	Fr	UC		X	_	\mathbf{X}	_		X	
52B	$\mathbf{E}\mathbf{x}$	UC			_	\mathbf{X}				
54	Fl	UC	(E)	_		X		_		
57	$\mathbf{E}\mathbf{x}$	UC	L	_		\mathbf{X}		_		
60A	Ex	UC	L		\mathbf{X}	X	_			
60B	$\mathbf{E}\mathbf{x}$	UC	L	X		?				
60C	$\mathbf{E}\mathbf{x}$	UC	L	X		X		_		
62C	Fr	UC			_	X	_			
67	Ex	UC	L	_		\mathbf{X}		_		
68	$\mathbf{E}\mathbf{x}$	UC	L	X		X	_	_		
69	\mathbf{Fr}	UC	L			\mathbf{X}	_			
70	$\mathbf{E}\mathbf{x}$	UC	_			\mathbf{X}		_		
76	Ex	UC	L	X	\mathbf{X}	X	_		-	
77	Fl	LC	L	_		X	_		_	
83	Fl	LC	(E)			X	_			
84	\mathbf{Fl}	LC	Ĺ	_	_	X		-	_	
9 2 A	Ex	UC	(E)		\mathbf{x}	X	_	_	X	
110	$\mathbf{E}\mathbf{x}$	UC	Ĺ	X		X				
115	$\mathbf{E}\mathbf{x}$	UC	L			\mathbf{X}		_	_	
119	Fr	UC		_		\mathbf{X}	_	_		
121	Fr	UC			_	X				
123	Ex	UC	${f L}$	_		X				
125	Ex	UC	L			X	_			
126	Ex	\mathbf{UC}				X	_			
133	Ex	UC	\mathbf{L}	_		X	-			
155	Fl	UC	(E)	X		X				
159	Fr	UC	<u> </u>	X		X	_		X	
177	Ex	UC	L	X		X			_	

KEY TO TABLE: Ex=extended burial; Fl=flexed burial; Fr=fragmentary burial; UC=upper cave; LC=lower cave; L=late, after 7000 B.P.; (E)=early, at 7000 B.P. or before, to 11,000 B.P.

The Niah Cave palaeoserology can be compared to the blood group data from modern populations in Borneo, particularly Sarawak. These data are given in Table 2, which is a summary table from the most recent edition of Mourant's world blood-group distribution tables (Mourant, Kopec, and Domaniewska-Sobczak 1976: 949, 1001).

TABLE 2. BORNEO ABO BLOOD GROUP SYSTEM FREQUENCIES BY AREA AND POPULATION

SYSTEM	N	NORTH :	BORN	ЕО	SAR	AWAK					вс	RNEO				
GROUP S	DU	suns	MU	RUTS		SEA YAKS	BR	UNEI	KED	AYANS		AND YAKS		EA ZAKS	MEL	ANAUS
вгоор	NO.	%	NO.	%	NO.	. %	NO.	%	NO.	%	NO	. %	NO.	%	NO.	. %
0	173	46.63	208	56.52	7	22.58	19	38.00	51	28.65	26	26.80	31	37.35	77	43.75
A_1	48	12.94	77	20.92	6	19.35	12	24.00	79	44.38	20	20.62	27	32.53	33	18.75
В	140	37.73	76	20.65	12	38.71	18	36.00	36	20.22	38	39.18	16	19.28	49	27.84
A ₁ B	10	2.69	7	1.90	6	19.35	1	2.00	12	6.74	13	13.40	9	10.84	17	9.66
Total	ls 371		368		31		50		178		97		83		176	

Source: After Mourant, Kopec, and Domaniewska-Sobczak 1976: 949, 1001.

The majority of population samples from Borneo show a higher, or at least an equal, frequency of blood group B to blood group A. When considering the small sample size of the palaeoserological data, the frequency of blood group A_1 is greater than that of B. This information may not be out of line when one considers the location of Niah Cave. According to the mapping of the gene A frequency for Borneo, this location is within the highest gene frequency area of the island. The palaeoserology may well be reflecting this serological distribution (Mourant, Kopec, and Domaniewska-Sobczak 1976: Appendix, Map 13).

RADIOCARBON DATING

There had been questions, prior to the sample testings by Geochron Laboratories, Inc., and Isotopes, Inc., as to whether the human bone recovered at Niah Cave could be radiocarbon dated. There were two possible difficulties, one that the bone might be sufficiently ancient so that permineralization processes had been initiated through time, preventing the utilization of either collagen or bone apatite, as these would have been replaced during the permineralization (Cook, Brooks, and Ezra-Cohn 1961). The other problem was that the heavy bird guano deposit lying over the burial areas, in combination with the moist atmosphere, especially toward the mouth of Niah Cave, would have leached out the collagen or bone apatite. Geochron Laboratories, Inc., decided to run a second test on the bone from Burial no. 60C as they felt that the "date on the carbonate fraction of bone is slightly too young

possibly due to exchange with CO₂ in the groundwaters and further that the date from the coffin material is slightly too old" (H. Krueger, personal communication, 4 August 1966). In Table 4 the second bone test, which was based on their radiocarbon dating of the organic material, is the C-14 date listed.

Table 3 lists the C-14 dating results of the 30 burials that the Rikagaku Kenkyusho laboratory analyzed, including C-14 dates in years B.P. based on the half-lives of 5730 and 5568 years. In the column labeled "Comments" they have identified the basis for the dating error as "collagen contents were found to be quite variable sample by sample as indicated in the list. The collagen-poor samples would have yielded less reliable dates because the effect of humus contamination would then be greater. Those having traces of collagen have been dated on bone apatite. The reliability of apatite date is still under discussion" (T. Hamada, personal com-

TABLE 3. RADIOCARBON DATA FROM NIAH CAVE

	C-14 DATE		
BURIAL NUMBER	HALF-LIFE 5730 YRS.	HALF-LIFE 5568 YRS.	COMMENT
3	1930 ± 80	1870 ± 75	A
10	$3420\ \pm\ 125$	3320 ± 120	В
30	$3820\ \pm\ 485$	3710 ± 470	C
36	$2880\ \pm\ 85$	2800 ± 80	A
50	2270 ± 160	2210 ± 150	C
54	$10,900 \pm 525$	$10,600 \pm 505$	D
57	2590 ± 135	2520 ± 130	В
60A	3040 ± 115	2960 ± 115	В
66	$7050~\pm~650$	6850 ± 600	D
67	2710 ± 85	2630 ± 80	A
68	3770 ± 105	3660 ± 100	Α
69	3260 ± 100	3170 ± 100	\mathbf{A}
75	2700 ± 390	2630 ± 375	D
76	4290 ± 90	4160 ± 90	A.
77	$3680\ \pm\ 75$	3580 ± 70	A
83	8230 ± 265	8000 ± 255	D
89	Modern		*
92	7350 ± 170	7140 ± 165	Α
102	2740 ± 85	2660 ± 80	A
110	5130 ± 90	4990 ± 90	Α
115	4780 ± 200	4650 ± 195	В
123	3590 ± 160	3490 ± 155	В
125	2810 ± 125	2730 ± 120	В
133	3060 ± 85	2980 ± 85	Α
135	2970 ± 405	2880 ± 390	D
146	11,700 + 1600	11,400 + 1550	D
	— 1400	— 1350	
147	7220 ± 140	$7020\ \pm\ 135$	A
148	565 ± 105	550 ± 100	*
155	8080 ± 180	7850 ± 175	В
177	2690 ± 85	2620 ± 80	Α

Source: Rikagaku Kenkyusho (The Institute of Physical and Chemical Research).

KEY: A = collagen rich; B = collagen medium; C = collagen poor; D = carbon in bone apatite dated; * = resin-like substance appears on surface.

munication, 24 March 1973). Table 3 is arranged by burial number rather than by antiquity. In Table 4 the first column contains an adjusted C-14 date range derived as a compromise between the two half-lives of radiocarbon, as listed in Table 3, and the standard errors. The adjusted C-14 date ranges are listed semichronologically in that where there is a large standard error, consequently a range overlapping occurs.

Discussion of the C-14 Dates

In the palaeoserological discussion it was mentioned that all extended burials occur in the Upper Cave, which concurs with the description by B. Harrisson (1967) in a paper detailing the distribution of various types of burial positions in Niah

TABLE 4. Chronology of C-14 Dates, Palaeoserology, Burial Position, and Sex Identification

adjusted c-14 date range	BURIAL NO.	BLOOD GROUP	SEX	BURIAL POSITION		
Modern	89		I	fragmentary		
л.р. 1325-1525	148	_	1	flexed		
A.D. 720	60(B)	$\mathbf{A_1}$	m	extended-C		
a.d. 40–110	3	$\mathbf{A_1}$	m	extended-C(?)		
160 в.с.	60C	A_1	f	extended-C		
520 в.с.	84	0	f	flexed		
350-100 в.с.	50	0	f	$\operatorname{extended-M}$		
1000-250 в.с.	75	-	f	extended-C		
650-450 в.с.	57	0	m	extended-M		
750-550 в.с.	67	0	m	fragmentary		
750-550 в.с.	177	A_1	I	fragmentary		
800-600 в.с.	102		f	extended-M		
1300-500 в.с.	135		I	fragmentary		
930-630 в.с.	125	0	f	extended-C(?)		
850-820 в.с.	36	0	${f f}$	extended-C		
1000-880 в.с.	60A	В	m	extended-C		
1100-900 в.с.	133	0	f	extended-M		
1300-1100 в.с.	69	0	f	fragmentary		
1450-1340 в.с.	10	$\mathbf{A_1}$	f	extended-C		
2000-1200 в.с.	30	0	I	fragmentary		
1670-1350 в.с.	123	0	f	extended-C		
1700-1500 в.с.	77	0	f	flexed		
1780-1580 в.с.	68	A_1	f	extended		
2300-2100 в.с.	76	A_1B	m	extended-C		
2800-2400 в.с.	115	0	m	extended-M		
3100-2900 в.с.	110	A_1	m	extended-M		
5500-4250 в.с.	66		I	fragmentary		
5200-4800 в.с.	147		m(?)	fragmentary		
5400-5000 в.с.	92	В	I	fragmentary		
6075-5675 в.с.	155	A_1	f	flexed		
6300-5700 в.с.	83	0 '	m	flexed		
9000-8000 в.с.	54	0	m	flexed and fragmentary		
11,030-8030 в.с.	146		Ī	fragmentary		

KEY: C = coffin; M = matting only; m = male; f = female; I = indeterminate.

Cave. Those burials that were radiocarbon dated are cited in the last column of Table 4 as to whether they were extended in a coffin or wrapped in matting, or whether they were flexed or incomplete and fragmentary. On the basis of the 33 burials that were radiocarbon dated, it appears that extended burials began about 3000 B.C. and continued through nearly to A.D. 1000. Both of the 2 late burials contained a resin-like substance, noted by Rikagaku Kenkyusho, and this may have affected the C-14 dating analysis. Flexed burials occur through time, but are more frequent in the earlier chronological sequence of the series selected for C-14 dating. Sex of the burials, where it could be identified, was included to determine if there might be any correlation between sex and burial position or burial in coffins or matting wrappings. No correlation is apparent in this selected burial series.

The burials selected for radiocarbon dating on the basis of the various factors described in the Introduction do demonstrate a steady use of Niah Cave over time, even into the relatively recent period. Some of the fragmentary burials were so incomplete that no cancellous bone or vertebrae were available to submit for palaeoserological analysis, especially Burials 66, 146, and 147, which are among the older burials radiocarbon dated. Blood groups A_1 , B, and O appear in the earlier series, although it is interesting that none of the burials more recent than about 800 or 900 B.C. have tested as blood group B, while 4 out of the 10 burials C-14 dated after this time are A_1 . This may simply reflect the size of the series analyzed palaeoserologically and C-14 dated, or it may relate to the higher frequency of the blood group A_1 gene recorded recently in this section of Borneo (Mourant, Kopec, and Domaniewska-Sobczak 1976).

SUMMARY

A selected series of 34 burials from the Great Cave of Niah has been analyzed palaeoserologically and 25 of these burials radiocarbon dated. The 33 burials chosen for radiocarbon dating also were selected to include burials which if dated might determine the chronological distribution of various burial practices or solve other archaeological problems. Extended burials in coffins or wrapped in matting do occur through time, beginning about 3000 B.C. and continuing to around A.D. 1000. Flexed burials are scattered through the time sequence of this series.

Blood group O has a frequency of occurrence of nearly 59% and dates from the earliest burials to a fairly recent time period. Blood group A_1 occurs the next most frequently—over 32%—and burials with this blood type are found relatively early and more recently than blood group O. The frequency of blood group B is low, 2 out of 34 burials, or about 6%, and there is $1\ A_1B$ blood type. Utilizing the A_1B type as an indicator of blood group B, the B blood group occurred in this small series as early as about $5000\ B.c.$, but no burials C-14 dated later than around $800\ to\ 900\ B.c.$ were palaeoserologically determined to be type B blood group.

The frequency of the A gene in recent populations in this area of Borneo is similar to that obtained in the palaeoserological research study, but the recent B and AB gene frequencies are higher, while the recent O gene frequencies are much lower. Additional studies correlating palaeoserology with C-14 dating from this section of Sarawak would be necessary before any far-reaching conclusions could be drawn as to the chronological significance of the blood groups of these burials

and their distribution through time. In the anthropometric and morphological analysis of the approximately 200 complete and incomplete burials from Niah Cave, presently being finished, this initial burial study will be amplified by correlation to both quantitative and qualitative skeletal data analyses. These analyses may answer some questions, but may equally raise even more problems.

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