

SEATO Medical Research Study on Malaria

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Period of Report: 1 April 1965 - 31 March 1966

General Information:

Related information to malaria studies can also be found under the studies on renal disease and on entomology.

Since the summer of 1965, there has been a very major increase in the number of malaria studies and the scope of interest. New studies include human clinical studies, non-human primate studies and the basic physiology and immunology of the parasites.

Also in the same period a study center has been established at Phrabuddhabat, an endemic area of malaria. In this area we have use of an animal house, insectary and laboratory space through the kindness of the National Malaria Eradication Project. Collaborative studies on patients are done with the staff of the Phrabuddhabat District Hospital.

STUDY REPORTS

1. Title: Malaria and the Nervous System
Ammonia Levels in Blood and Spinal
Fluid in Cerebral Malaria and Malarial Hepatitis

Principal Investigators: Martin Chipman, Major, MC.
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Period of Report: 1 October 1965 - 31 March 1966.

Objective: Measure venous blood and spinal fluid ammonia in hospitalized patients without malaria, with uncomplicated malaria, and with malaria complicated by nervous system and liver involvement.

Description: In our recent study² at Phrabuddhabat Hospital we found four patients with cerebral symptoms who also had evidence of acute malarial hepatitis. In all cases neurological improvement closely paralleled decrease in liver size and the disappearance of liver tenderness.

The role of ammonia as a factor in hepatic encephalopathy is controversial. Although some studies have been unable to find correlations between ammonia levels and liver and brain dysfunction, others have reported high blood and spinal fluid ammonias in hepatic coma associated with hepatocellular degeneration and with the shunting of portal blood around the liver.

Procedure: All patients admitted to the Phrabuddhabat Hospital with a blood smear positive for malaria were examined by the investigators and divided into four following groups:

1. Uncomplicated malaria.
2. Malaria with nervous system symptoms.
3. Malaria with hepatic symptoms (enlarged, tender livers and scleral icterus.)
4. Malaria with both nervous system and hepatic symptoms.

A fifth group of non-malaria hospital patients and blood donors were included in the study.

Method of collection: Blood. Three ml. of blood were drawn from the ante-cubital vein, placed in a cold EDTA-treated test tube, stoppered, and shaken. The test tube was then placed in a beaker of dry-ice in water or water in cracked ice and immediately taken to the laboratory where determinations were started approximately five minutes after drawing.

Blood was collected from patients before breakfast and between 0700 and 0800 the morning after admission.

Spinal fluid. 1 to 1.5 ml of CSF was placed in a cold tube, stoppered, placed in an ice bath and again immediately taken to the laboratory for ammonia determinations.

Notes

1. Physician, Phrabuddhabat Hospital, Saraburi Province, Thailand.
2. Chipman, M., Cadigan, F., and Benjapong, W., "Malaria and the Nervous System. Clinical Experience with a Hospital Population in an Endemic Area in Thailand."

Determination of Ammonia: The method used is a modification of the Conway microdiffusion technique. Figure 1 illustrates the microdiffusion dish and arrangement of reagents in the dish. After preparing the microdiffusion dish the following procedures are carried out:

- (A) Allow to stand three hours at room temperature.
- (B) Use "Spectronic 20" cuvettes and proceed as follows:
 - 1. 0.4 ml solution from center well.
 - 2. 1.0 ml phenol color reagent.
 - 3. 1.0 ml alkali hypochlorite reagent. Cover with parafilm and mix by inversion.
- (C) Place tubes in water bath at 37°C for fifteen minutes.
- (D) Add 2.0 ml of distilled water, mix thoroughly and
- (E) Read O.D. at 640 mμ $\frac{\text{O.D. unknown}}{\text{O.D. Standard}} \times \text{Conc. Std.} = \text{ug}\%$

Note If color is too intense, dilute with distilled water and read again correcting for added dilution.

Discussion of Data: McDermott, Adams, and Riddell (1955) used the Conway microdiffusion technique to measure peripheral blood (presumably venous) and cerebrospinal fluid ammonia levels. In their study, the blood ammonia levels in twenty normal controls ranged from 44-71, ugms% with a mean of 55.6 ugms%. The normal cerebrospinal fluids in seven cases ranged from 0-14.7 ugms% with a mean of 6.4 ugms%. In twenty-two cases with chronic liver disease and or portal hypertension, the blood ammonia levels varied from 66-326 ugms% with a mean of 254 ugms%; spinal fluids ranged from 16-213 ugms% with a means of 85 ugms%.

Refer to Tables 1 and 2 for the presentation and a simple analysis of our blood and cerebrospinal fluid ammonia data.

Superficially, there appears to be a significant difference between the mean blood ammonia levels in patients without malaria and those with malaria and nervous system symptoms; however, the wide range in normal values and the very high standard deviations in all groups raises questions of the significance of the data.

At this time, then, no valid statements can be made about ammonia levels in blood and cerebrospinal fluid in our five groups of patients.

General Information: A future study designed to investigate blood and CSF ammonia levels in malaria patients should incorporate the following changes in method:

- 1. Blood should be drawn both from an artery and from the internal jugular bulb and levels of ammonia compared in each
- 2. Stricter control should be exercised on the transport and processing of the samples.

Addendum:

In order to determine the range of values in a single sample five simultaneous measurements of blood ammonia were done on three consecutive days on two normal individuals (B and C). The methods of collection and determination were the same as described in the body of the text. Table 3 lists the information obtained.

FIGURE 1. THE CONWAY MICRODIFFUSION DISH

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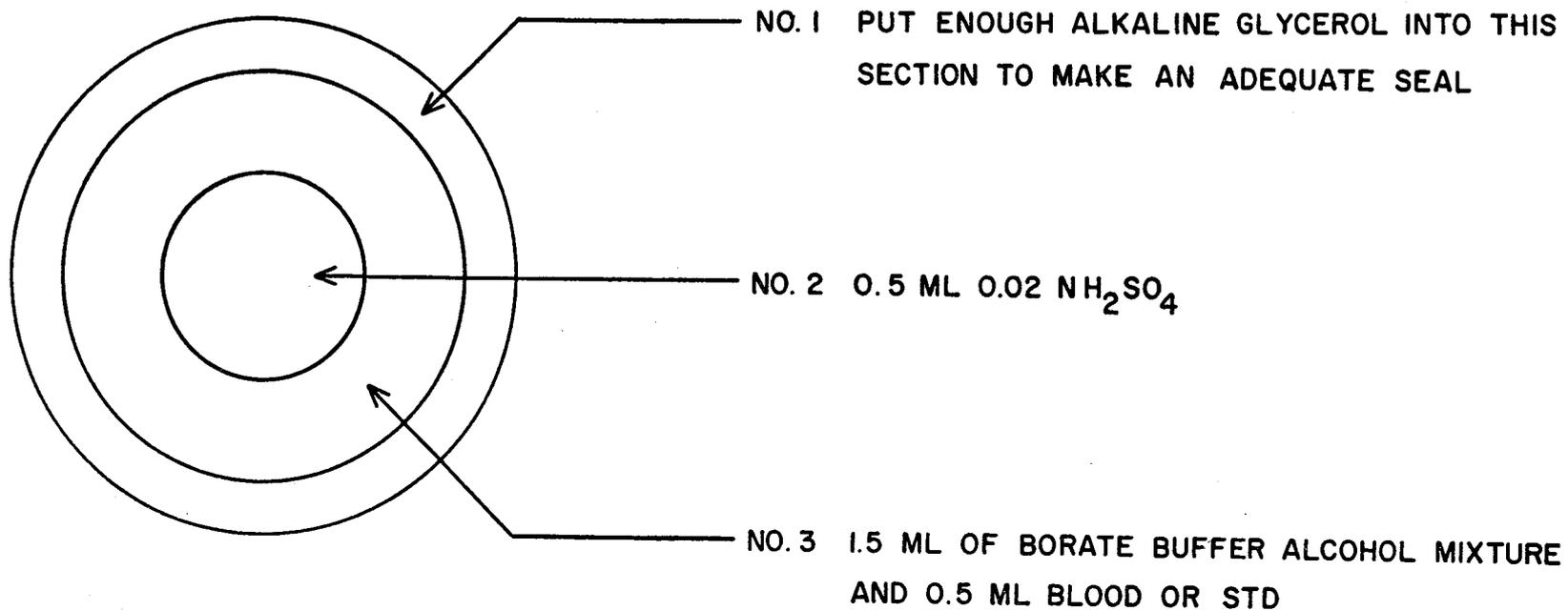


TABLE 1

Blood and Cerebrospinal Fluid Levels in
Malaria and Non-Malaria Patients

No.	Non-Malaria		Malaria Uncomplicated		Malaria with Nervous System Symptoms		Malaria with Hepatic Symptoms		Malaria with Hepatic and Cerebral Symptoms	
	Blood ugms%	CSF ugms%	Blood ugms%	CSF ugms%	Blood ugms%	CSF ugms%	Blood ugms%	CSF ugms%	Blood ugms%	CSF ugms%
1	36	17	27	1	107	4	63	8	158	52
2	37	25	33	4	108	8	63	8	158	52
3	39	33	33	4	108	11	65	16		
4	45		50	8	113	11	75	16		
5	54		54	8	140	13	90	20		
6	54		54	9	142	13	101	24		
7	54		61	13	163	16	109	33		
8	58		66	16	166	19	126	40		
9	58		66	17	166	27	131	45		
10	63		68	20	167	36	135	135		
11	71		73	21	168	43	143			
12	75		75	34	175		177			
13	76		77	85	186		179			
14	76		78	86	190					
15	79		83		207					
16	79		85		215					
17	80		87							
18	82		90							
19	87		90							
20	87		100							
21	91		104							
22	91		108							
23	91		108							
24	93		115							
25	95		116							
26	100		118							
27	116		120							
28	116		133							
29	125		133							
30	125		136							
31	134		137							
32	140		150							
33	140		158							
34	143		165							
35	145		169							
36	147									
37	150									
38	152									
39	154									
40	166									
41	171									
42	233									
43	266									

TABLE 2

Analyses of Blood and Spinal Fluid Ammonia Data

	Mon-Malaria Patients		Malaria Patients, Uncomplicated		Malaria Patients with Nervous System symptoms		Malaria Patients with Hepatic Symptoms		Malaria Patients with Hepatic & Cerebral Symptoms	
	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF
Number	43	3	35	14	16	11	13	10	2	2
Range of Values	36-266 ugm%	17-33 ugm%	27-169 ugm%	1-86 ugm%	167-215 ugm%	4.43 ugm%	63-179 ugm%	8-135 ugm%	158-183 ugm%	52-75 ugm%
Mean	104 ugm%	25 ugm%	95 ugm%	23 ugm%	158 ugm%	19 ugm%	112 ugm%	35 ugm%	---	---
Standard Deviation	49	---	52	23	35	12	41	38	---	---

Table 3

Data on Multiple Determinations of Blood Ammonia Done
on Three Consecutive Days on Two Normal Subjects (B and C)

Subject	Date	Blood Ammonia (ugms%)					Optical Density					Range of Blood Ammonia (ugms%)	Mean ugms%	Standard Deviation
		1	2	3	4	5	1	2	3	4	5			
B	26 April 1966	118	109	107	106	103	.120	.111	.109	.108	.105	103-118	109	5.7
	27 April 1966	172	166	180	199	—	.182	.175	.190	.210	—	166-199	179	14.4
	28 April 1966	101	114	158	180	162	.115	.130	.180	.205	.185	101-180	143	114
C	26 April 1966	98	107	88	96	97	.100	.109	.090	.098	.099	88-107	97	6.8
	27 April 1966	109	104	101	183	142	.115	.110	.107	.193	.150	101-183	128	35
	28 April 1966	123	105	123	177	—	.140	.120	.140	.262	—	105-177	132	33