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Field Trials of Biochemical Blood Examination in Relation to Chemotherapy of Malaria Patients in Palawan Island, the Philippines

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ABSTRACT : During the courses of chemotherapeutic research of falciparum and vivax malaria patients in a rural area, some biochemical examinations of blood and urine samples were tried to carry out by using a portable electric generator, a digital blood analyser kit, and a small D. C. power supply for electrophoresis. Comparisons were made between samples, as a rule, just before starting radical treatments with chloroquine or other drugs and after completion of two-weeks' follow-up. (1) Quantitative analyses were done for Hb, total protein, albumin, GOT, GPT, LDH, blood urea nitrogen, zinc sulphate turbidity test, etc. Increased levels in LDH, GOT and GPT, and decreased levels in total protein, albumin, and Hb were noticed in both falciparum and vivax malaria patients before the drug treatment. (2) Results of polyacrylamide gel electrophoresis showed an improvement (increase) of prealbumin level after the chemotherapy in almost all the patients. Decrease in an unidentified protein at post-transferrin position was noticed after the cure of malaria in almost all the falciparum cases but in about half of vivax cases. (3) Analysis of LDH isozyme patterns in plasma indicated that severe hemolysis of the host RBC was mainly responsible for the elevated LDH levels in malaria patients. Difficulty in assorting haptoglobin types for many malaria patients also supported this explanation by severe hemolysis. (4) Remarkable improvements in urine protein and urobilinogen levels were observed after the drug treatment, whereas no recognizable alteration was found in pH, glucosuria, and bilirubin levels. (5) By thin-layer chromatography on precoated silica gel plates, it was quite easy to detect antimalarial drugs extracted from urine samples of patients at various intervals even one week after the oral administration, but the detection of chloroquine from blood samples was not successful.

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Informations on blood protein and enzyme levels or their electrophoretic patterns of a patient are quite useful for diagnosis in some diseases, and these techniques have now become routine in most of clinical laboratories. With respect to the course of human malaria, the infection may influence, either directly or indirectly, protein levels of patients, and various investigators have reported such alterations in blood biochemical values as shown in Table 1. Most of these works have been carried out under well-facilitated laboratory conditions, and some cases involve results of even experimental infections of human malaria to human volunteers or to other primates. Under the circumstances of field survey on malaria in actual tropical zones, however, it is rather difficult to obtain necessary informations on the clinical biochemistry in each patient before and after starting chemotherapy with an antimalarial drug. Therefore, the preliminary trials were attempted to carry out some clinical blood examinations and plasma electrophoresis using a portable electric generator in a field condition or in a poorly facilitated rural hospital.

Table 1. Reported alterations on serum (or plasma) biochemical levels due to infections with human malaria

Item	Alteration	Malaria	Host	Reference
Total protein	Increase	f	Thai	Sadun *et al.* (1966)
	Decrease	f	American	〃
	〃	f	Chimpanzee*	〃
	Decrease	f	Aotus	Wellde *et al.* (1972)
Prealbumin	Decrease	f, v	Human	Tsukamoto *et al.* (present paper)
Albumin	Decrease	v	Human	Taylor *et al.* (1949)
	Decrease	f, v	Human	Lunn *et al.* (1966)
	Decrease	f	Human	Sadun *et al.* (1966)
	〃	f	Chimpanzee*	〃
	Decrease	f	Aotus	Wellde *et al.* (1972)
Glycoprotein in albumin fraction	No change	f, v	Human	Klainer *et al.* (1968)
α_1-Globulin	Increase	v	Human	Taylor *et al.* (1949)
	Increase	f, v	Human	Lunn *et al.* (1966)
	Increase	f	Thai	Sadun *et al.* (1966)
	No change	f	American	〃
	Decrease	f	Chimpanzee*	〃
α_1-Glycoprotein	Increase	f, v	Human	Klainer *et al.* (1968)
〃 (mainly α_1-Antitrypsin)	Increase	f, v	Human	Murphy *et al.* (1972)
$\alpha_1+\alpha_2$-Globulin	Increase	f	Human	Collins *et al.* (1971)
α_2-Globulin	Decrease	f, v	Human	Lunn *et al.* (1966)
	Decrease	f	American	Sadun *et al.* (1966)
	No change	f	Thai	〃
	Increase	f	Chimpanzee*	〃
α_2-Glycoprotein	Decrease	f, v	Human	Klainer *et al.* (1968)
〃 (mainly Haptoglobin)	Decrease	f, v	Human	Murphy *et al.* (1972)
Haptoglobin	Decrease	f, v	Human	Blumberg *et al.* (1963)
β-Globulin	Increase	v	Human	Taylor *et al.* (1949)
	Increase	f	Human	Sadun *et al.* (1966)
	Decrease	f	Chimpanzee*	〃
β-Glycoprotein	No change	f, v	Human	Klainer *et al.* (1968)
β_{2M}-Macroglobulin (=19S, IgM)	Increase	v	Human	Abele *et al.* (1965)
Complement	Decrease	f, v	Human	Dulaney (1948)

C3	No change	m	Human	Cameron & Kibukamusoke (1971)
Fibrinogen	Decrease	f	Human	Devakul *et al.* (1966)
	Increase	f	Human	Areekul *et al.* (1971)
	Increase	f	Human	Fletcher *et al.* (1972)
Fibrin degradation products	Increase	f	Human	Jaroonvesama (1972)
γ-Globulin	Increase	v	Human	Taylor *et al.* (1949)
	Increase	v	Human	Kuvin *et al.* (1962)
	Increase	f, v	Human	Lunn *et al.* (1966)
	Increase	f, v	Human	Collins *et al.* (1971)
	Increase	f	Human	Sadun *et al.* (1966)
	〃	f	Chimpanzee*	〃
	Decrease	f	Aotus	Wellde *et al.* (1972)
γ_2-Globulin (7S)	Increase	v	Human	Abele *et al.* (1965)
IgM, IgA, IgG	Increase	v	Human	Tobie *et al.* (1966)
	Increase	f, v	Human	Collins *et al.* (1971)
γ-Glycoprotein	Increase	f, v	Human	Klainer *et al.* (1968)
Bilirubin	Increase	f	Human	Mukherjee *et al.* (1971)
BUN	Increase	f	Human	Goldstein (1968)
	Increase	f	Human	Canfield *et al.* (1968)
	Increase	f	Human	Stone *et al.* (1972)
	Increase	f	Aotus	Wellde *et al.* (1972)
Glucose	Decrease	f	Aotus	Wellde *et al.* (1972)
LDH	Increase	f	Aotus	Schnell *et al.* (1969)
GOT	Increase	f	Human	Goldstein (1968)
GOT, GPT	Increase	f	Human	Sadun *et al.* (1966)
	Increase	f	Chimpanzee*	〃
	Increase	f	Aotus	Schnell *et al.* (1969)
	Increase	f	Aotus	Wellde *et al.* (1972)
	Increase	f	Human	Willerson *et al.* (1974)
AlkP	Decrease	f	American	Sadun *et al.* (1966)
	No change	f	Thai	〃

* Splenectomized

The works reported here have been carried out in Palawan Island, the Philippines, from October 1971 to January 1972, and from January to February 1973, by members of the Nagasaki University Medical Research Team, in which the last author was the team leader, in cooperation with members of the Malaria Eradication Service, Department of Health, Republic of the Philippines.

Materials and Methods

Patients. All the out-patients who visited the General Hospital, Iwahig Penal Colony, Palawan, were at first screened for malaria by examining blood smears during the period of our survey. Patients (♂♂) confirmed microscopically to be infected with either *Plasmodium falciparum* or *P. vivax*, were admitted into the hospital at the Central area or an infirmary at the Montible area. A total of 118 patients consisting of 63 vivax, 49 falciparum, 2 malariae cases and 4 of mixed infections (with *P. vivax* and *P. falciparum*) was treated with drugs and was under the follow-up observations in the 1971-1972 survey. Patients who showed

recrudescence were omitted from the test group. Among them 53 were subjected to the biochemical blood examinations (23 falciparum cases and 30 vivax cases). In the 1973 survey, a total of 220 malaria patients (212 falciparum, 7 vivax, and 1 mixed cases) was treated, and 74 falciparum patients were subjected to the chemotherapeutic research.

Chemotherapy. Radical treatment with chloroquine diphosphate (Resochin or Aralen, 150 mg base/tablet) was carried out to the patients as previously reported by Nakabayashi *et al.* (1974). Sulfamethopyrazine (Policydal, 100 mg/tablet) was also used, 800 mg at Day 0, followed by each 100 mg for Day 1 to Day 4, alone or in combination with chloroquine. For the convenience of explanation, a typical course of the drug treatment, follow-up, blood smear examination, and biochemical examination was shown in Fig. 1.

Clinical Examination of Blood. The examinations were done as a rule twice for each patient, i. e., the first just before starting the drug treatment and the second after follow-up observation when the parasite detection on thick blood smears continued submicroscopic at least for two weeks.

Each 5 ml sample of blood was drawn from the cubital vein by using commercially available vacuum blood collecting tubes which contain appropriate amounts of EDTA • 2Na. Particular cares were paid to avoid any artificial hemolysis during all the procedures. An aliquot of blood was immediately examined for the hemoglobin (Hb) level. Plasma was then separated from blood cells by a centrifugator and was kept in an ice-cold thermo bottle. An aliquot of plasma was used for biochemical examinations within 2 hours and the rest was fur-

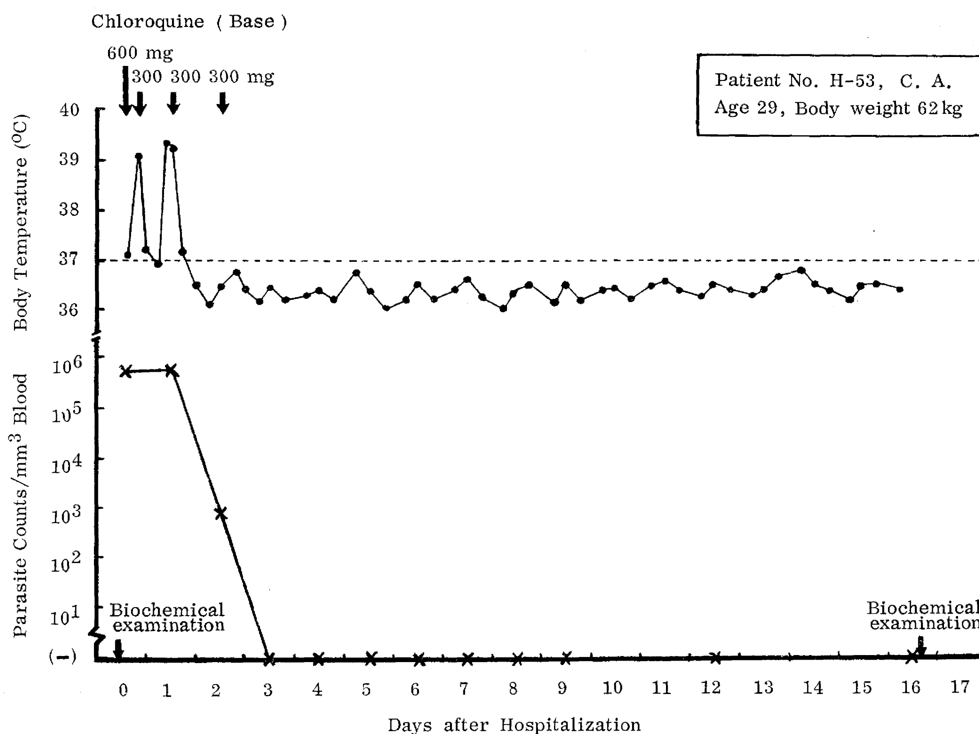

Fig. 1. Chemotherapeutic course with chloroquine diphosphate in a falciparum malarial case.

ther kept in a kerosene-operated refrigerator until used for electrophoretic analysis. As a control group, 10 healthy Filipinos who had no malarial experience at least for recent 5 years were chosen.

A digital handy blood analyser (UNIKIT-RaBA system, Chugai Pharmaceutical Co., Ltd.) connected with a portable electric generator (Honda, 300W, 100V) was used for the clinical-biochemical analysis. Chemical reagents to be used for this kit were kept at room temperature (about 30℃ in the day-time and about 20℃ at night). The items tested were: hemoglobin (Hb), total protein (TP), albumin (Alb), aspartate aminotransferase (glutamate-oxaloacetate transaminase, GOT), alanine aminotransferase (glutamate-pyruvate transaminase, GPT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), leucine aminopeptidase (LAP), alkaline phosphatase (AlkP), and zinc sulfate turbidity test (ZTT).

Clinical Examination of Urine. At the same time, urine of the patients was also examined by using a commercially available simple reagent strip (Hemacombistix, Ono Pharmaceutical Co., Ltd.) for pH, glucosuria, occult hematuria and proteinuria. In addition to these items, handy color tests for urobilinogen and bilirubin (Sino test No. 5 and No. 6, respectively, Sino-test Laboratory) were also employed.

Electrophoretic Analysis of Plasma. Horizontal electrophoresis on 5% polyacrylamide gel plate was carried out to compare the patterns of plasma protein and some enzymes before and after the chemotherapeutic course. The practical method employed was essentially same as described by Tsukamoto (1974). The portable electric generator operated by kerosene was connected to a small D. C. power supply directly or through a half-down transformer. Under these conditions the voltage was usually within a range of 120−150V during the electrophoresis. The plasma samples to be examined were kept in a refrigerator except for the LDH pattern analysis which was carried out with fresh samples within several hours after plasma separation because LDH-5 and LDH-4 bands were less stable than other isoenzyme bands during the storage.

After the electrophoretic run, proteins were simultaneously fixed and stained with 0.5% Amido black 10B in a mixture solution of methanol−water−acetic acid (5 : 4 : 1, v/v), and destained in 7% acetic acid. The gels destained or stained for enzyme activity bands were sandwiched with cellophane sheets and the resultant dried films were kept. The electrophoretic patterns were analysed by a scanning densitometer (Joko Sangyo Co.) in the laboratory after comming back to Japan, and the densitometric data were further analysed by a computor. In a similar way, detection of enzyme activity band(s) was tried for LDH, LAP, AlkP, and esterases. Peroxidase activity of Hb associated with benzidine-nitroprusside staining (Ogita, personal communication) was utilized for determining haptoglobin (Hp) types of individuals.

Detection of Antimalarial Drugs from Urine Samples. After the chemotherapy with an antimalarial drug, about 50−75 ml of fresh urine samples of patients were extracted with chloroform after alkalification with a conc. NaOH solution. The chloroform-extractable fraction was washed by a diluted NaOH solution and chloroform was evaporated in air. The organo-soluble residue was then analysed by thin-layer chromatography on a pre-coated TLC silica gel plate (E. Merck) in an empty instant coffee bottle. A solvent system employed

was methanol—acetic acid—water (4: 5: 1 or 4: 6: 1, v/v). Another solvent system of chloroform—acetic acid—water (1: 6: 1) was also employed in some cases. For detecting chloroquine or amodiaquine on a TLC plate, Dragendorff reagent was sprayed after ascending chromatography about 7—9 cm. For detecting sulfamethopyrazine, Ehrlich reagent was used.

RESULTS AND DISCUSSION

I. Quantitative Analysis of Blood Biochemical Levels

As mentioned above, comparison on quantitative data on some biochemical examination was made on admission day to the hospital (namely, just before starting the chemotherapy for malaria) and on discharge day from the hospital (namely, after completion of the chemotherapy and the subsequent follow-up). Table 2 summerizes plasma levels of various items (except for Hb test where a whole blood sample was used in the analysis). At first, much more patients were subjected for these examinations, but during the course of the follow-up, unnegligible numbers of patients were omitted from the counts by various reasons, such as recrudescence of parasitemia (resistant cases), transfer of colonists to other subcolony or colony, release from the colony (completion of the sentence), and so on. The final number of the falciparum case was 23, among them 17 were treated with chloroquine and 6 were with sulfamethopyrazine. Although the final number of subjects was too small, there were no remarkable difference in blood biochemical data between these drug groups or between vivax and falciparum cases.

Biochemical reagents to be used for blood examination are usually requested to be stored in the dark and cold place such as in a refrigerator, and under the usual laboratory condition there is no difficulty to do so. In a field medical research at a rural area of the tropical zone, however, it is rather impossible to find out an appropriate cold room to keep

Table 2. Comparison of plasma biochemical levels (average and range) between data of pre- and post-chemotherapeutic treatments

Items tested	Unit	Falciparum malaria (23 cases)		Vivax malaria (30 cases)		Normal control (10 cases)	
		Before treatment	After treatment	Before treatment	After treatment	First examination	Two weeks later
Hb*	g/dl	12.8 (9.4—15.6)	13.5 (10.8—15.2)	13.3 (8.1—15.5)	13.8 (6.8—15.6)	15.5 (12.9—17.9)	15.2 (13.2—16.9)
TP	g/dl	7.0 (5.6—7.7)	7.4 (6.4—8.6)	7.0 (5.9—8.3)	7.4 (5.9—8.0)	7.4 (6.8—8.2)	7.3 (6.9—8.0)
Alb	g/dl	4.1 (2.3—5.2)	4.5 (3.5—5.1)	3.9 (2.6—5.2)	4.4 (3.6—5.8)	5.1 (4.5—5.7)	4.9 (4.4—5.3)
BUN	mg/dl	31.1 (25.1—40.1)	26.6 (22.6—29.2)	29.7 (22.8—36.8)	28.0 (22.7—31.5)	24.0 (20.7—29.5)	27.7 (25.1—29.8)
ZTT	Kunkel	2.8 (1.7—4.3)	3.5 (1.8—5.8)	3.0 (1.2—4.5)	3.5 (2.4—5.2)	1.9 (0.8—3.0)	2.2 (1.0—3.5)
LDH	Wroblewski	426 (200—730)	326 (150—520)	380 (120—820)	256 (120—420)	161 (220—380)	201 (180—320)
GOT	Karmen	22 (12—47)	18 (9—49)	28 (10—89)	21 (10—49)	15 (12—18)	16 (8—26)
GPT	Karmen	24 (12—74)	17 (12—35)	24 (8—90)	21 (14—53)	20 (11—37)	16 (13—22)

* Whole blood samples were used exceptionally for the Hb test.

large quantities of chemical reagents for several months. Therefore, some simulation tests were also performed in our laboratory, Nagasaki University, to examine whether or not these chemical reagents could be stored at tropical room temperature. A group of chemical reagents was kept in a could room at 3−8℃ as usual, and the other in an incubator adjusted to 30℃. Blood examinations of healthy subjects (members of the team) were carried out at various intervals by using reagents of both groups. Contrary to our initial expectation, no remarkable difference was observed between data obtained by using reagents of these groups when the original seal of the reagents was kept unopened up to 5 months. Exceptions were the cases of LAP and AlkP, where the data examined by chemicals kept at 30℃ always gave us higher measurements than those in the control groups. The data for LAP and AlkP are thus omitted from Table 2.

II. Clinical Examination of Urine

Urine samples of patients were also examined for pH, protein, sugar, Hb, bilirubin and urobilinogen. Practically no alteration was observed in urine pH, and all the patients showed negative results in the glucosuria and hematuria tests. Of 45 patients whose urine gave positive results in the urobilinogen test on admission, 31 subjects showed improved changes in urobilinogen test after the chemotherapy (16 of 26 urobilinogen test positive patients in vivax malaria, and 15 of 19 positive tests in falciparum malaria). Similar improvements were observed in the urine protein test: of 36 positive subjects 29 showed improved results (18 of 21 in vivax cases and 11 of 15 in falciparum cases). Table 3 presents comparisons of actual data for urine protein and urobilinogen between pre- and post-chemotherapy tests. It is quite obvious that the distribution patterns have shifted towards negative in accordance with symptomatic improvement of these patients.

III. Electrophoretic Analysis of Plasma Proteins

Comparisons in electrophoretic patterns of proteins were carried out between plasma samples taken from the same individuals just before and after the chemotherapy. Human blood plasma (or serum) is composed of a number of proteins and separable into several amido-black stainable bands after electrophoresis (Fig. 2).

Prealbumin: Normal range of prealbumin is quite lower than that of other major plasma

Table 3. Alteration of protein and urobilinogen levels in urine samples of malaria patients after chemotherapy (expressed as number of patients)

Color test		Falciparum malaria (19 cases)		Vivax malaria (30 cases)	
Item	Grade	Before treatment	After treatment	Before treatment	After treatment
Protein	−	5	13	10	22
	±	8	6	11	7
	+	5	0	8	1
	++	1	0	1	0
Urobilinogen	−	2	4	5	11
	±	2	6	7	10
	+	2	7	4	7
	++	3	2	6	2
	+++	10	0	8	0

proteins, and the separation of prealbumin from albumin is not achieved by usual cellulose acetate electrophoresis. These are major reasons why quantitative variation of prealbumin has often been ignored in routine clinical examinations. In the present work, however, an increase in prealbumin level after the antimalarial treatment (and the subsequent follow-up for two weeks) was evidently noticed by polyacrylamide gel electrophoresis of plasma samples in almost all the malaria patients. This was further confirmed by scanning densitometry (Fig. 2): namely, in the first examination just before starting the chemotherapy, an average prealbumin level was 22.5 mg/dl (range, 16−39 mg/dl) in 13 falciparum cases, and 16.2 mg/dl (range, 6−40 mg/dl) in 15 vivax cases. After the chemotherapy, on the other hand, the average levels increased to 31.2 mg/dl (range, 22−44 mg/dl) in falciparum malaria, and to 27.4 mg/dl (range, 15−40 mg/dl) in vivax malaria, respectively. These increases in prealbumin level after chemotherapy were statistically highly significant. As far as we know, the decrease in prealbumin level has not been reported in connection with malaria. Although the present observations are not based on experimental infections, and although it is not sure whether or not such an increase in prealbumin level is a temporary phenomenon after the cure from severe symptoms of malaria, it might be said that the decrease in prealbumin level is one of the characteristic phenomena in human malaria.

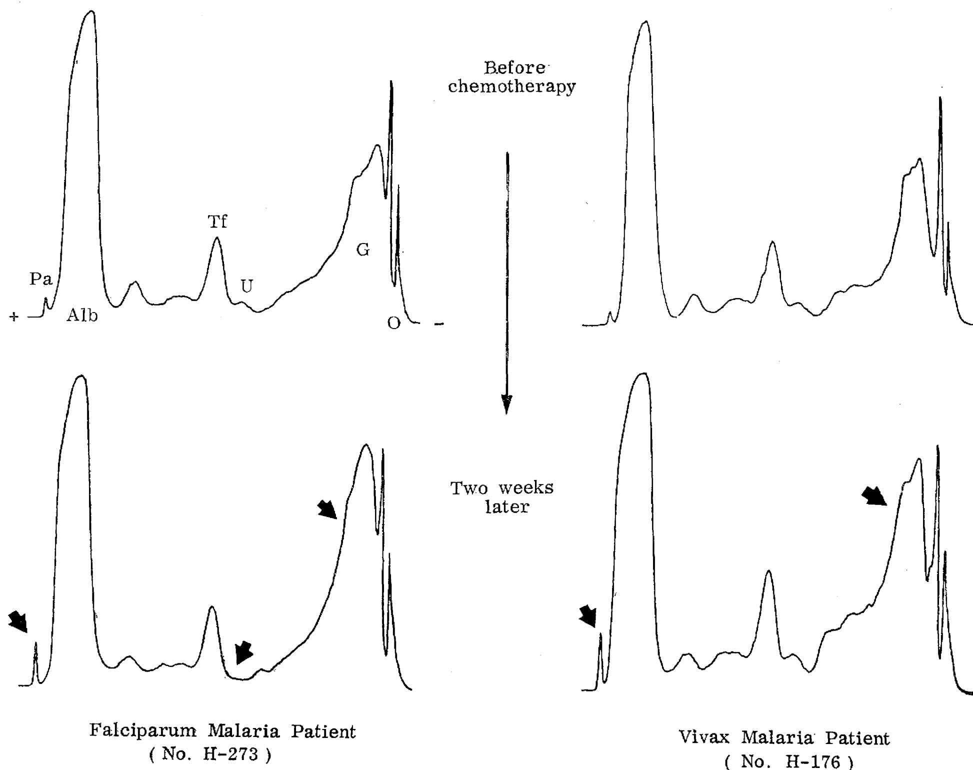

Fig. 2. Electrophoretic comparison between plasma protein patterns before and after the chemotherapy in the same patients. Note the increase or decrease in some protein fractions indicated by solid arrows.
Pa: prealbumin, Alb: albumin, Tf: transferrin, U: unidentified, G: gamma-globulin zone, and O: origin.

Prealbumin is known as a carrier of thyroid hormones and of vitamin A (Kanai *et al.*, 1968; Raz *et al.*, 1969). Thus the increase in prealbumin level of the malaria patients after the chemotherapy might reflect the accelerated rate of general metabolism after the recovery from the severe illness.

Unidentified Protein Band: In most falciparum cases, quantitative alterations of an unidentified protein fraction with a slightly slower electrophoretic mobility than transferrin were observed; namely, electrophoretic patterns showed a rather high level of this fraction in plasma taken from patients before the chemotherapy whereas this protein decreased after the drug treatment. In vivax malaria, on the other hand, such a decrease in this protein was observed among only a half of the patients, and this protein fraction still remained high among the other half of the vivax patients even after cure of the malaria. At present it is not yet clear whether this change is characteristic to the falciparum cases or has occurred by chance.

The protein itself is, in some cases, recognizable even in fresh plasma samples, and hence such an alteration in this protein region may not be due to a "storage effect" such as a change of β_{1C}-globulin to β_{1A}-globulin, whereas the electrophoretic position of this protein band is still suspicious enough to lead a temporary identification as β_{1A}-globulin fraction. If this is the case, the reason is still unknown why the different results have been obtained between these two malarial groups. β_{1C}-globulin or the third component of complement (C3) is easily converted into a series of conversion products, among which β_{1A}-globulin (C3c) is the major one, and this conversion is related to immune hemolysis. In connection with malaria, Dulaney (1948) reported decreased complement contents in induced malaria patients, whereas Cameron and Kibukamusoke (1971) could not show statistically significant differences in C3 levels between malariae malaria patients and controls. One of another possibility for the identification of this unknown protein is one of the Hb-Hp complex bands. It is well known that Hb easily binds to Hp and forms several kinds of Hb-Hp complexes of which electrophoretic mobilities are different from each other or from that of unbound Hp molecule. The electrophoretic behaviour of the Hb-Hp 1−1 band is sometimes similar to that of the protein band in question, and it is also known that the mobility of the Hb-Hb complex bands is changeable in accordance with the degree of saturation with Hb (Ogawa *et al.*, 1968). Thus the possibility that this unidentified protein band is an intermediate Hb-Hp complex still cannot be neglected.

Gamma-globulins: Increasing levels in gamma-globulin zones were observed in accordance with results by other investigators (Fig. 2, Table 1).

Haptoglobin Type: In the case of malaria patients, severe hemolysis occurs in the blood stream in accordance with the life cycle of erythrocytic forms of malaria parasites, and in spite of a scavengering activity by Hp, excess amounts of Hb or its related complexes or degradation products might be one of the major causes of clinical symptoms. It is also known that 2−2 type Hp binds less Hb than Hp 1−1 or Hp 1−2 (Curtain *et al.*, 1965), and a selective advantage of Hp^1 gene in malarious tropical areas is still an attractive explanation, although they could not find any significant difference in Hb type distribution between malaria parasite positive and negative groups. For these reasons, Hp has attracted our attention, too; then blood samples of some malaria patients were examinied for Hp typing by

electrophoresis.

Out of 34 malaria patients examined, 4 belonged to Hp 1−1 type, 8 were Hp 1−2 type, and 8 were Hp 2−2 type. However, another 14 could not be identified because of absent or faint Hp band. In the case of healthy persons as controls, Hb-Hp patterns were quite typical and hence Hp type of individuals could be easily deternimed. Among Filipino populations, Blackwell *et al.* (1963) reported Hp type distribution and the frequency of Hp^1 gene was 0.38. Similar results on the distribution of Hp types (the gene frequency of Hp^1: 0.41) and difficulty in identifying Hp types among pathologic subjects were also shown by Bayani-Sioson (1968) in 473 normal and 109 patients of the Filipinos, whereas no malaria case was included in her report. Concomitant decreases in Hb and Hp levels were demonstrated by Blumberg *et al.* (1963) during the course of experimental infection of malaria to volunteers. Murphy *et al.* (1972) reported that a decrease in α_2-glycoprotein in malaria was mainly due to a decrease in Hp. The present study confirmes the decrease in Hp in malaria patients more directly.

LDH: It has been mentioned in earlier part of the present paper and in Table 2 that plasma LDH levels of malaria patients are sometimes extremely high in spite of the greatest possible caution to prevent or minimize technical hemolysis of erythrocytes (RBC) after blood taking. By electrophoresis the LDH enzyme can be separated usually into 5 isoenzyme bands. Therefore it may be possible to know by examining the isoenzyme pattern whether such an abnormally high LDH level in plasma of malaria patients is accountable for possible hepatic lesions or for severe disintegration of RBC by malaria parasites in blood stream. Relative color intensity of LDH isoenzymes after nitro bule tetrazolium-staining in normal plasma is:

$$\text{LDH-2} > \text{LDH-3} > \text{LDH-1} > \text{LDH-4} \gg \text{LDH-5}$$

and the isoenzyme pattern of normal hemolysates is:

$$\text{LDH-1} > \text{LDH-2} > \text{LDH-3} \gg \text{LDH-4} \gg \text{LDH-5}.$$

In the cases of plasma of malaria patients, on the other hand, the increase in LDH−1 isoenzyme activity was significant, and the order of the color intensity is:

$$\text{LDH-1} \geqq \text{LDH-2} > \text{LDH-3} > \text{LDH-4} \gg \text{LDH-5}.$$

These findings evidently indicate that the increased level of LDH−1 band in malaria patients is originated mainly from the disintegration of RBC but not from that of liver cells. In some cases, of course, the LDH patterns were indicative of the presence of some hepatic lesion, but the intensity of LDH−2 or LDH−1 band was far superior to that of other isoenzyme bands.

Presence of an extra LDH isoenzyme band of the parasitic origin was not observed in plasma of malaria patients over the period of the present field survey whereas in the case of a rodent malaria an extra LDH isoenzyme band was detected in plasma of malaria-infected mice (Tsukamoto, 1974). Carter and McGregor (1973) reported that some human blood samples infected with *P. falciparum* showed the presence of additional 3 enzyme bands of the parasitic origin other than usual human LDH patterns. They did not mentioned, unfortunately, whether the parasite enzyme bands were found in patient's plasma or hemolysates.

Miscellanea: In parallel with works on the protein pattern, electrophoretic analysis of so-called "non-specific" esterases, LAP, and AlkP activity bands was also attempted. No

remarkable or unique pattern was observed in comparison of plasma between normal and malarial individuals, between falciparum and vivax patients, or between pre- and post-chemotherapeutic samples.

IV. Detection of Antimalarial Drugs from Urine Samples after Chemotherapy

Several cases of drug-resistant malaria have been experienced in the research area during the period of the present survey as previously reported (Nakabayashi *et al.*, 1974). Apart from genetic character of the malaria parasites, slow and low absorption or rapid excretion of an orally administrated antimalarial drug by patients might also be one of suspicious causes for so-called "drug-resistance". In more extreme case, the treated amount of drug is not absorbed physiologically or psychologically (for example, by vomitting the tablets) into the patient's blood stream and the oral treatment with an antimalarial drug does not show any curative effect; thus in such a case a physician might easily be misled to be as if a "drug-resistance" case. Actually, we have also had such experiences with some malaria patients. Therefore, attempts were made to detect a given drug from patients' blood or urine samples. After extraction of drug (and its metabolites, if any) from biological samples with chloroform, the organo-soluble residue was analysed by ascending chromatography using a pre-coated silica gel G plate. Chloroform was chosen as an extracting solvent in field works because of its non-inflammability, high volatility, easy availability, multi-purpose usability as an anesthetic for mosquito examinations or as a developing solvent in thin-layer chromatography, and so on.

From blood samples, the detection of chloroquine or amodiaquine was not successful at various time intervals after the oral radical treatment. On the other hand, the administrated drug was easily detected from urine samples even 2 hours after the oral treatment, suggesting that some parts of the drug are rapidly absorbed into patient's blood system and soon excreted into the urine. Usually, even up to 7 or 10 days after the radical treatment with chloroquine or amodiaquine, the presence of relatively large amounts of the drug could be easily detected from urine samples by TLC. Thus, a possible misleading due to no or poor absoption can be exculded from so-called "drug-resistance" cases in malaria by employing such a simple TLC method in field.

CONCLUSIONS

From the results obtained from such preliminary field works, it is rather inadequate to draw any definite or precise conclusions on clinical-biochemical data as being expected in a well-facilitated laboratory. It has been demonstrated, however, that such blood analysis are not only possible even in field but also effective for well-understanding patients' conditions of illness during the course of therapy and follow-up.

In tropical zones, except for urban or suburban areas, availabilities in electricity, water supply and transportation are usually quite limited. Such inconveniences might be overcome to a certain extent by using a portable generator, ice-box, kerosene- or propane-operated refrigerator, and so on. Recent development of handy clinical examination kits made it possible to carry out blood examination easily in field. In such cases most of the chemical rea-

gents are requested to be stored in a cold place, and this seemed to cause a difficulty in practical application in field works. Results of the present paper, however, suggest that even without a cold room or a large refrigerator such a clinical blood analysis can be done on many items which are most commonly employed in usual clinical laboratories. Results on some of enzyme tests, for example LAP and AlkP, have been omitted in the present paper, because they gave us abnormally higher values after storage at tropical room temperature for few months. It might be, however, possible to estimate adequate values by adjusting data from a calibration curve, or by improving constituents of the chemical reagents in future.

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フィリピン・パラワン島辺地におけるマラリア患者血液の臨床生化学的検査

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1971年から1973年にかけてフィリピン・パラワン島イワヒグ地区においてマラリア患者の化学療法とクロロキン耐性についての研究を行なったが，その際患者の血液（および一部尿についても）の臨床生化学的検査を試みた．当地区は電気，水道，ガスなどの設備のない辺地であるが，携帯用の小型発電機や灯油冷蔵庫などを利用することにより有用な知見を得ることが出来たので，その結果の概略について報告する．抗マラリア剤（主としてクロロキン）投与による同一マラリア患者について，通常治療前および治癒後の２回静脈血を採取し，血漿分離後種々の検査成績について比較した．治療前には，三日熱・熱帯熱いずれの場合にも総蛋白，アルブミンなどの量が低下していたが，治癒後増加が見られた．また，乳酸脱水素酵素（ＬＤＨ），アスパラギン酸アミノ転移酵素（ＧＯＴ），アラニンアミノ転移酵素（ＧＰＴ）などの活性値はマラリア患者血漿では高い者が多かったが，治癒後は低下した．ポリアクリルアミドゲル平板による患者血漿の電気泳動ではプレアルブミン量が低かったが，治癒後殆んど全部の患者で増加が認められた．プレアルブミンはビタミンＡや甲状腺ホルモンの担体として知られているので，マラリア治癒後の体内での活発な代謝促進を反映しているものと解釈できる．また患者血漿中にはトランスフェリンよりやや泳動度の低い未同定の蛋白バンドがあるが，熱帯熱マラリアの場合には治癒後減少する傾向が認められたのに対し，三日熱マラリアの場合は治癒の前後とこの蛋白量の増減との間に一定の関係は見られなかった．マラリア患者では上記の如く血漿ＬＤＨ活性値が異常に高い者が多いが，その原因を知るための電気泳動によるアイソザイム分析の結果，肝臓障害由来と考えるよりはむしろマラリア感染による赤血球の著しい溶血に由来するものであると判断された．患者の症状の軽重とハプトグロビン（Hp）型との関係の有無を知る目的で予備的調査を行なったが，マラリア患者では型判定の困難な場合が多かった．これも多量の溶血に伴なうHpの血流中での著しい減少に基づくものと解釈すれば説明がつけられる．血液以外にも患者尿についていくつかの検査が行なわれたが，蛋白やウロビリノーゲンなどの量は治癒後有意に減少することが示された．また経口投与後のクロロキンは，２・３時間から１週間にわたって患者尿中から薄層クロマトグラフィーで容易に検出されたが，血液中からの検出は困難であった．

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