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Effects of Pregnancy on *Trypanosoma congolense* Infection in Rats :

Serum Biochemistry and Cellular Disorders

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Résumé

L'évolution de l'infection provoquée par le *Trypanosome congolense* chez des rates gravides semble très différente de celle constatée dans le cas de rates non-gravides.

La moyenne des différences des charges de trypanosome (par millimètre cube de sang en circulation) entre les colonies de parasites présentes chez les rates gravides et non-gravides contaminées est statistiquement élevée.

Les variations du nombre de globules blancs et de globules rouges traduisent les augmentations et les diminutions qui se produisent en réaction au besoin physiologique changeant de l'hôte et des cellules de trypanosome elles-mêmes.

Il ressort du niveau élevé de BUN obtenu lors des tests biochimiques du sérum des rates gravides parasitées que les parasites sont probablement moins sensibles à l'action uréique.

Les rates gravides atteintes du *Trypanosome congolense* ont montré qu'elles possédaient une quantité réduite de protéine sérique, de sodium et de calcium ainsi qu'une concentration assez normale de potassium.

Les tests biochimiques du sérum montrent aussi qu'un état d'hypoglycémie et d'hypercholestérolémie subsiste chez les rates parasitées, gravides et non-gravides.

Ces résultats confirment l'existence de troubles physiologiques dans le sang de l'hôte parasite. Ils suggèrent également que la

légère déviation trouvée dans les ions de potassium et les valeurs non équilibrées des électrolytes de sodium et de chlorate sont étroitement liées à la virulence de la maladie qui comporte probablement des inhibitions du transport actif de ces ions.

Les changements pathologiques observés dans les globules seuls ou accompagnés de modifications métaboliques du sérum détectées chez les hôtes qui ont été exposés au trypanosome sont concomitants de l'apparition des symptômes cliniques. Les implications de l'état gravide ou de la régulation hormonale de l'hôte pendant la trypanosomiase sont décrites dans le document.

Summary

The course of *Trypanosoma congolense* infection in pregnant rats appears to be very different from that of non-pregnant infections.

The mean of the differences in trypanosome burdens (per cubic millimeter of circulating blood) between the parasite populations present in the pregnant and non-pregnant infected rats is statistically signifiant.

The changes reported in WBC and RBC counts strongly reflect increases and decreases in response to the changing physiological requirement of the host and the trypanosome cells themselves.

The higher level of BUN obtained from the serum biochemical tests of pregnant infections indicates that the parasites are probably less susceptible to Urea action.

The pregnant rats infected with *Trypanosoma congolense* showed reduced amount of total serum protein, sodium, calcium, and a somewhat normal potassium concentration.

The serum biochemical tests further indicate that conditions of hypoglycemia and hypercholesterolemia persisted only in the pregnant and non-pregnant infected rats.

These results confirm the existence of physiological disturbances in the infected host's blood The results also suggest that the slight deviation found in potassium ions and the unbalanced values of sodium and chloride electrolytes are interrelated to the disease virulent mechanism which probably involves inhibitions of active transport of these ions.

The pathological changes observed in ihe blood cells alone or together with the serum metabolic alterations detected in the hosts after exposure to trypanosome infection are both concomitant with the appearance of the clinical symptoms. The implications of pregnance state or hormonal regulation in the host during trypanosomiasis are hereby documented.

Introduction

One recognized fact in the studies of human health and parasitic diseases is the interaction between veterinary medicine and human medicine. This interaction has grown with increasing complexity, particularly in relation to public health problems in the developing nations. Consequently, parasitologists, clinicians, biologists and parabiochemical researchers have discovered, through their ever greater dependence upon experimental works with animals, that their field of activity and interests should no longer be only to gain knowledge useful for comparative medicine and biological functions. Therefore, for human benefits ; increasing efforts now are limitlessly extended towards discovering or finding better diagnostic methods, prevention, eradication, inhibition of growth or developments and treatment of parasitic diseases.

While substantial progress on controls continues, more research efforts seem to be the only answer to the yet unsolved problems, especially on the pathogenic group of trypanosomes. Emmet (1950), Thillet and Chandler (1957), and Sanders and Wallace (1966) have demonstrated that rats immunized with irradiated *Trypanosoma lewisi* became immune to a challenge inoculation with normal trypanosomes. Passive immunity against *Trypanosoma gambiense* in mice was also reported by Seed and Gam (1966). Petana (1965) reported that cortisone treatment aggravated the course of infection of *Trypanosoma congolense* in albino rats. In addition splenectomy state, irradiation, Berenil compounds have produced resistance to *Trypanosoma congolense* in rats and mice (Hawking 1963, Irfan 1968). However, Dunn and Brown (1962) claimed that natural levels of all hormones during pregnancy and lactation neither enhance or inhibit the infection of pin worms (*Aspiculuris tetrapetra* and *Syphacia obvelata*) in mice, while Dunsmore (1966) observed that increases in the susceptibility of pregnant rabbits to the nematodes (*Trichostrongylus retortaeformis* and *Graphidium strigosum*) was greatly influenced by reproductive hormones.

In view of these earlier reports and the existing problems on African trypanosomiasis, it was of interest to examine further the host-parasite implications of *Trypanosoma congolense,* its behavior and physiological function in a « conditioned » environment. The degree of parasitemia in pregnant and non-pregnant hosts together with its detail serum biochemistry and pathology are carefully considered. We believe that the information presented in this report will be of paramount values in understanding better the pathogenicity of human trypanosomes, blood parasites and related diseases.

Materials and methods

The albino rats used in this study were from the parasite free inbred colony maintained in our Parasitology laboratory, animal house, University of Ife. Several sets of male and female rats were mated so that we could get enough pregnant rats for the experiments. The pregnant and the non-pregnant females when infected were

10 weeks old. The strain of *Trypanosoma congolense* used was the same as in previous studies (Simaren 1969, 1970 *a*), and had been maintained in normal albino rats in our laboratory by syringe passage of saline suspensions of infected rat tail blood over the past 5 years. Trypanosomes from 6 days old stock infection were used throughout this work. The method for separating and preparing the pure cells needed for infection was that of Lincicombe and Watkins (1963). Then the separated trypanosome cells were calculated to contain the desired number needed for experimental infections.

Group I consisted of 16 pregnant rats, each intraperitoneally inoculated with 4 000 *Trypanosoma congolense*. Group II consisted of 16 non-pregnant rats similarly infected with 4 000 trypanosomes. Group III which composed of 16 non-pregnant rats, and Group IV made up of 16 pregnant rats were not infected. These two groups served as controls. All rats were separately caged and were fed Purina laboratory chow and water *ad libitum*. Trypanosome and blood cell counts were performed chow and water ad libitum. Trypanosome and blood cell counts were performed daily beginning the 5th day following infections. The quantitative method, for determining the levels of parasitemia in the circulating blood of infected hosts and for the red and white blood cells count with the aid of hemacytometer, Thoma pipette and Toisson's fluid followed the same pattern previously reported (Simaren and Bammeke 1970 *d*).

The biochemical determination conducted in the study were tests mainly for serum levels of total protein, glucose, blood urea, nitrogen, cholesterol, sodium, potassium, calcium and chloride. These tests were performed on the day after infection. Each blood was allowed to clot before centrifugation, after which the serum was removed. Total serum protein was qualitatively determined (Henry et al. 1957, Lowry et al. 1957). Glucose values were measured following the techniques of Folin-Wu (1920, 1921 ; Tonks 1952). Tests for Blood Urea Nitrogen (BUN) were performed by the method of Sobel et al. (1944), while the techniques of Zak (1957) were used for the determination of total cholesterol level. Measurements of serum sodium levels were conducted by the method of Albanese and Lein (1948).

Analysis for potassium and calcium were made by the modified methods of Lockhead and Purcell (1951), Clark and Collip (1925), Fawcett and Wynn (1961) directly using Coleman flame photometer and titrations. The chloride determinations were performed by automatic titration with « direct read out » of chloride concentration on the Buchler-Cotleve chloridometer. The chloridometer measures the amount of silver ions precipitated as silver chloride on the silver coated electrodes (Cotlove and Nishi, 1961).

Result

The sixty-four albino rats used in the 4 group experiments were thoroughly examined daily for trypanosome counts beginning with the 3rd day after inoculation. Summarized data showing the average incubation period, the degree of parasitemia,

peaks of infection, variabilities in RBC and WBC counts, rate per cent multiplication or increment in trypanosome population per day and the mean change of biochemical values in tests performed on sera from the rats are presented (Tables I-IV, fig. 1-4).

The course of the decrease varied. Group I pregnant infected rats reproduced a different orderly pattern of parasitemia compared with the non-pregnant Group II. No differences occurred in the prepatent period in all the experiments. The calculated trypanosome burdens increased from a low level of 2.1 thousands on day 5 to a peak of 1090 thousands on the eleventh day for Group I. Mean population for the 7 days period was 542.6 thousands. In Group II experiments, trypanosome cells multiplication rose from 1.1 thousands to 560 thousands for the same period with a mean population of 254.3 thousands. Mean difference between the two trypanosome populations in the experiments I and II was 288.3 thousands (Table I).

A steady quantitative increase in WBC and a gradient fall in RBC were observed in Groups I and II between the 5th and fifteenth day (Table II). Population rate of RBC depletion and WBC increment was much slower in the uninfected pregnant control than in the non-pregnant control (Table II). Group I rats which were not necropsied did not survive beyond the thirteenth day post infection.

No significant changes in the biochemical values for potassium and calcium on sera from the infected (pregnant and non-pregnant) and the uninfected (pregnant and non-pregnant) animals (Table IV).

Tests indicate lower values for sodium in the infected pregnant and non-pregnant groups, and the uninfected pregnant animals (Tables III-IV). Elevated serum chloride determinations were recorded for all the rats tested in all groups (Tables III-IV). The sodium chloride balance in the sera from the pregnant infected animals was 403 mg. per 100 ml. while the value for the non-pregnant infected rats was 366 mg. per 100 ml. In the uninfected pregnant and non-pregnant controls, the serum sodium chloride balance were 378.4 per 100 ml. for the former, and 378.4 mg. per 100 ml. for the latter.

In the pregnant infected animals of Group I, the value of serum BUN (blood urea nitrogen) obtained were higher and almost as twice than normal. But the level in the non-pregnant infected rats showed no significant deviation from those of the uninfected controls.

Cholesterol levels in the sera of rats in Group IV were also recorded higher than the controls. However, an unusual contemporaneous increase of chloride and cholesterol levels were found in the sera of pregnant infected and non-pregnant infected animals compared with the non-pregnant uninfected controls (Tables III-IV).

Decreased values for total serum protein and glucose were prominently detected in sera of rats from Group I and II compared, between Group I and IV, and between Groups II and III (Tables III-IV).

Mean differences in levels of calcium, chloride, BUN, cholesterol, total protein and glucose levels were significant between the pregnant infected animals and the pregnant uninfected groups (Table III).

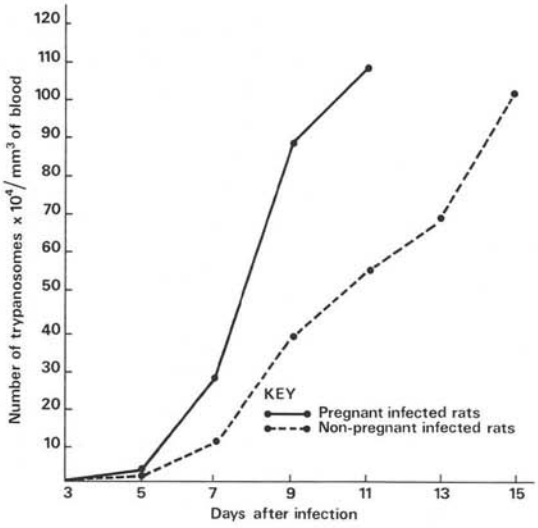

FIG. 1

Table I. — Population of Trypanosomes in Pregnant and Non-pregnant rats infected with 4000 *Trypanosoma congolense*. Data are expressed in thousands

Group Experiments	I			II		
Rat's Condition	Pregnant			Non-pregnant		
No. of Rats/Group	16			16		
Parasitemia/mm^3 Blood	Population		Increment Rate %	Population	Increment Rate %	Difference between I and II Populations
Days after Infection						
5	2.1		—	1.1	—	1.0
6	125.3		5820	47	4154	78.3
7	270		100	111	134.7	159
8	498		84.7	265	129.9	233
9	890		78.7	380	43.3	510
10	923		4.0	416	9.5	507
11	1090		18.1	560	34.9	530
Total in 7 days ..	3798.4			1780.1		2018.3
Mean	542.6			254.3		288.3
Confidence Limit (95 %)	80.87	495.78				

Table II. — Mean population of RBC and WBC in the peripheral blood of pregnant and non-pregnant rats infected with 4000 *Trypanosoma congolense*.
(Cell Counts are expressed in thousands)

Group experiments condition of rats Number of Rats Per Group	Infected Rats		Uninfected Rats	
	I Pregnant 16	II Non-pregnant 16	IV Pregnant 16	III Non-pregnant 16
RBC/mm³ : Days				
5	887	1020	1840	1920
7	692	1009	2037	1890
9	620	940	1990	1724
11	420	952	2080	1923
13	*	832	2110	1860
15	*	711	2310	1972
WBC/mm³ : Days				
5	4.78	4.98	4.84	5,12
7	6.53	6.02	6.30	4.30
9	7.50	7.11	6.67	4.87
11	6.48	6.62	7.34	5.50
13	*	7.55	8.65	7.53
15	*	6.42	6.50	4.60

* Autopsied

Table III. — Serum Biochemical Effetcs of Infection on Pregnant and Non-pregnant Rats. Infected Rats Received 4000 *Trypanosoma congolense*

Group Experiment Rat's Condition	Pregnant			Non-Pregnant		
	I Infected	IV Uninfected	Mean Difference Values	II Infected	Uninfected	Mean Difference Values
No. of Serum/Rat	8	8		8	8	
Calcium mg/100 ml	12.33	18	5.67	13.33	17	3.67
Potassium mg/100 ml	41.72	39.7	2.02	42.13	38.5	3.63
Sodium mg/100 ml	256	261.7	5.7	224	273.4	49.4
Chloride mEq/2 ..	147	117	30	142	105	37
BUN mg/100 ml .	87.16	44.4	42.76	38.14	43.1	4.96
Cholestérol mg/100 ml	247.46	217.7	29.76	328.6	79.58	249.02
Total Protein mg/100 ml	35.8	47.7	11.9	45.45	49.32	3.87
Glucose mg/100 ml	7.7	35	27.3	13	36.7	23.7

Table IV. — Serum Biochemical Effect of Pregnancy on Infected and Uninfected Rats. Infected Rats Received 4000 *Trypanosoma congolense*

Group Experiments Rat's Condition	Infected			Uninfected		
	I Pregnant	II Non-Pregnant	Mean Difference in Values	IV Pregnant	III Non-Pregnant	Mean Difference in Values
No. of Serum/Rat	8	9		8	8	
Calcium mg/100 ml	12.33	13.33	1.0	18.00	17.00	1.0
Potassium mg/100 ml	41.72	42.13	0.51	39.70	38.50	1.2
Sodium mg/100 ml	256	224	32.	261.70	273.40	11.70
Chloride mEq/L ..	147	142	5	117	105.	12
BUN mg/100 ml .	87.16	36.14	49.02	44.40	43.10	1.3
Cholestérol mg/100 ml	247.46	328.60	81.14	217.70	79.58	138.12
Total Protein mg/100 ml	35.80	45.45	9.65	47.70	49.32	1.60
Glucose mg/100 ml	7.70	13.00	5.3	35.00	36.70	1.70

Discussion

Ashcroft (1960) and Simaren (1970 *a*) commented on the changeable properties of trypanosomes, their biological behavior and their extreme variations in susceptibility resulting in lethality of the infected host. As far as it is known, the whole mechanism by which these plasma dwelling organisms lower the functional capacity of the reticulo-endothelial systems prior to exerting their debilitating fatal effect on the host are not fully understood. The results of these studies and the eight separate biochemical tests on each group-experiment indicate that significant alterations occurred in the sera of pregnant and non-pregnant rats infected with *Trypanosoma congolense*.

During the period when the *Trypanosoma congolense* were in the peripheral blood, the rate per cent increment in trypanosome populations were calculated as incubation progressed. In the pregnant and non-pregnant infections, multiplication rate per cent of the parasites per day were found initially high, with more rapid degree in the former than in the latter (Table II). For the remaining incubation days, daily rate increment of trypanosome cells showed a declining phenomenon (fig. 2). A progressive steady rate of development was indicated between days 7 and 10 in Group I when a maximum height of parasitemia was attained (fig. 1 and 2). Group II, on the contrary, showed a closely identical rate per cent of trypanosome development between 7th and 8th day followed by an abrupt downward trend (fig. 2).

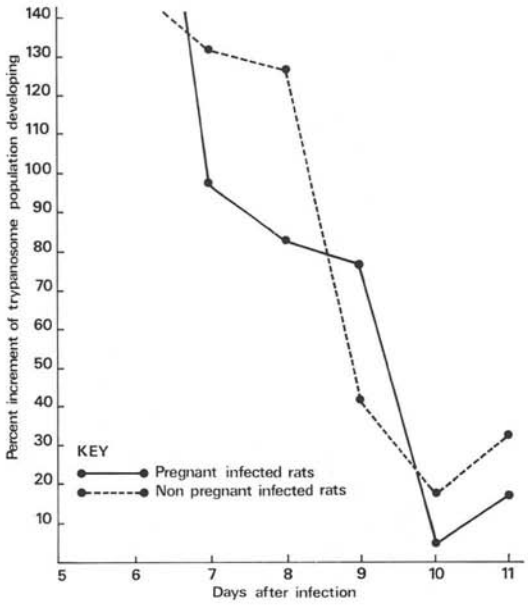
Percent increment of trypanosome population developing
140
130
120
110
100
90
80
70
60
50
40
30
20
10
KEY
Pregnant infected rats
Non pregnant infected rats
5
6
7
8
9
10
11
Days after infection

FIG. 2

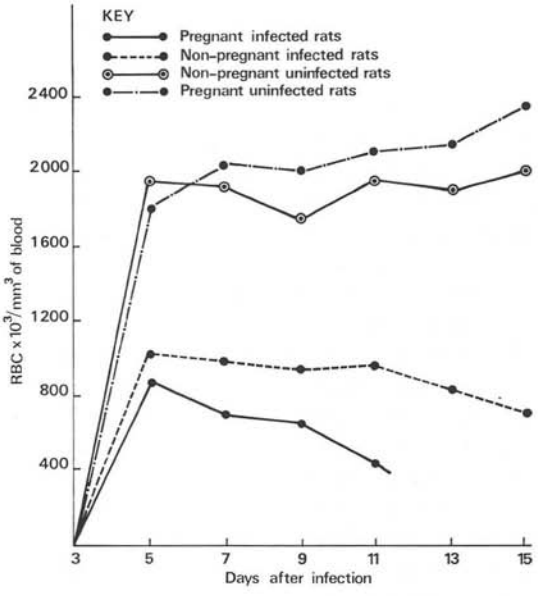
KEY
Pregnant infected rats
Non-pregnant infected rats
Non-pregnant uninfected rats
Pregnant uninfected rats
RBC x 10³/mm³ of blood
2400
2000
1600
1200
800
400
3
5
7
9
11
13
15
Days after infection

FIG. 3

Total number of trypanosomes produced by the pregnant infected rats was 2 millions greater than the non-pregnant infected group. The results also indicate that as infection progressed, more significant decline in RBC population with simultaneous numerical increases were observed in WBC of the pregnant infected rats than in the non-pregnant infections compared with the uninfected non-pregnant controls (Table II). Irfan (1986) reported that the spleen of infected intact mice with *Trypanosoma congolense* was 5 times enlarged than normal size. Simaren (1970 *a*) also reported that increases in splenic weights were shown by rats concurrently infected with *Trypanosoma congolense* and *Nippostrongylus brasiliensis* in one instance,

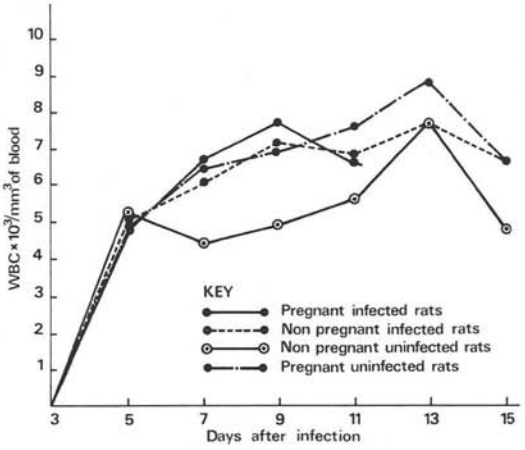

FIG. 4

and *Trypanosoma brucei* and *Nippostrongylus brasiliensis* in another experiment than by those singly infected. In the present investigation, the WBC numerical increase continued until the host's death. This part of the report supports the above findings. Irfan (1968) observed that the changes in WBC could have been aided by the increased phagocytic activity of the spleen which produced hyperplasia. It is known that during normal pregnancy the levels of hormone progesterone in mice and rats remain higher than that of estrogen in the blood. Since pregnant animals were used in our investigation, progesterone would therefore be the hormone most likely to exert a physiological influence on the parasite in these hosts. However, Dunsmore (1966) and Bull (1959) observed rises in egg counts of *Ostertagia* species in ewes and worm burdens in female rabbits (for *Trichostrongylus restortaeformis* and *Graphidium strigosum*) during the breeding periods. They postulated that the rise to high levels might be largely controlled by the host-hormones. The data we obtained in this work is parallel to their findings ; First, the results of our investigation in experiments I and II showed considerable differences in the daily rate per cent increases as well as total means for the trypanosomes population during the incubation period.

Secondly, we found that the daily rate at which the RBC decreases and WBC increases differed. Since these differences obtained in the means are statistically significant and the results support the hypothesis that the reproductive hormones of the host influence the host-parasite relationships ; It is suggested that the usually present higher level of blood progesterone existing in pregnant rats influenced or contributed to these changes observed in the cells and the trypanosome burdens.

During the second week of incubation, a significant decrease in the level of serum glucose of pregnant and non-pregnant infected host was detected. Increases in the total serum protein levels were also observed in the pregnant infections. The total serum protein level decreased slightly in the sera of the non-infected and pregnant uninfected groups compared with the non-pregnant uninfected controls (Tables III and IV). This significant departure from the normal values observed in the controls suggests a common pathogenesis of the host's parasitized blood. The picture may be indirectly reflecting protein deficient intake, synthesis or destruction as the disease progressively becomes severe.

Sadum *et al.* (1965), reported a reduced amount of total protein and glucose in mice infected with spargana of cestode *Spirometra mansonoides*. Their observations were summarized as moderate increases in serum total protein of mice exposed to *Schistosoma mansoni* infections for 10 weeks. Moon *et al.* (1968), reported slight increases in serum protein in mice infected with *Trypanosoma duttoni* and *Trypanosoma rhodesiense*. They claimed that the insignificant change discovered was due to the fluctutations in parasitemia in the mice. Sanchez and Dusanic (1968) observed hypoglycemia in rats infected with *Trypanosoma lewisi* when parasitemia was greatest. But under a different experimental condition of rats concurrently infected with *Nippostrongylus brasiliensis* and *Trypanosoma congolense*, hypoglycemic condition and serum changes in albumin and Beta-globulin detected were recently reported (Simaren and Bammeke 1970 *d*). These findings are in correlation with the consistent results obtained for glucose and protein in the present report.

However Weimer *et al.* (1959), noticed that serum enzyme levels in rats which had been previously starved during repletion dropped below normal values. They suggested that this was due to the fact that during repletion there is always greater synthesis of tissue protein resulting in less release of somatic enzyme, and therefore a drop in serum enzyme levels. A similar explanation may be occurring in this case, but since high parasitemias were produced in pregnant rats during the infections a critical factor which might have aggravated the blood physiological alterations is the unusual sugar demand and consumption of *Trypanosoma congolense*.

Elevated levels obtained for BUN tests in the sera from the pregnant infected rats were significant in comparison with the uninfected. Potassium concentrations were found slightly higher in the infected experiments than in the uninfected groups. Nevertheless, calcium levels in the pregnant and non-pregnant infections decreased than the values obtained for the uninfected controls (Tables III-IV). These findings for calcium (unlike that for potassium) are in agreement with the report of Moon *et al.* (1968), for *Trypanosoma rhodesiense* and *Trypanosoma duttoni* in mice, whereas the

elevated changes we obtained for BUN tests contradict their report. It is possible that the BUN result may be an indirect effect of pregnancy, indicating that the parasite are less susceptible to Urea action.

In Groups II and III, tests for sodium and chloride produced similar sodium chloride balance. Unbalanced differences of these electrolytes occurred in Group I, but the value appeared higher in Group I than the other groups except that the least sodium chloride value was found in Group II. This discrepancy could have been the result of depletion of salt especially since these ions are associated in diet.

The marked increases revealed in serum cholesterol in the pregnant and non-pregnant infected groups could be attributed to no other cause than the existing pregnancy state during the infections. It is interesting to note that no reasons were given for the low and variable blood cholesterol values reported by Kopp and Solomon (1943) for malarious human subjects and monkeys.

In conclusion, the occurring metabolic changes seemed to be direct manifestations or influences of pregnancy, parasitism and virulence of *Trypanosoma congolense* infection. These detected differences may be useful clinical diagnostic markers foi distinguishing pathogenicity between individuals that harbour and are not harbouring trypanosomes, any blood parasite and or others who may be moderately or chronically parasitized.

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