GROUP I.—(Cases not showing Pigmentation.)

Series.	Below 30 per cent.	30—40 per cent.	40—50 per cent.	50—60 per cent.	60—70 per cent.	70—80 per cent.	80—90 per cent.	90—100 per cent.	Total.
For both Races ...	...	0·78	3·53	12·55	36·47	36·08	10·59	...	100·00

SUMMARY.

1. *Hæmoglobin Index.*—No coolies arrived with a hæmoglobin index over 90 per cent. nor under 30 per cent.

The greatest number in any class were those showing a hæmoglobin index of 60—70 per cent. (37·76 per cent.) Both races favoured this.

2. *Certain forms of Helminthiasis.*—Only 6 individuals out of 331 were not proved to suffer from helminthiasis of one of the forms sought for.

Both races were found to be infected with the three diseases, the percentage returns for each disease not varying widely with each race.

3. *Pigmentation of the Papillæ of the Tongue.*—Pigmentation of the Papillæ of the sides and tip of the tongue is shown to be associated with ankylostomiasis in 90 per cent. of cases in these series.

4. *Anæmia* is a more marked feature in cases showing pigmentation of the tongue than in those which do not show it.

5. For the benefit of the existing forces on Rubber Estates, it is essential that all newly recruited coolies be examined for helminthiasis and treated if found necessary, in order to *attempt to check increase of existing infection at the threshold of the Estate.*

6. There is sufficient evidence to warrant *the routine treatment* of every cooly recruited from the Indian and China coasts.

7. Further evidence of coolies presenting anæmia to the degree shown, leads one to believe that it would be wise to reject at the port of emigration all those showing a hæmoglobin index under 60 per cent. and to view with suspicion all coolies so affected as to present the pigmentation of the tongue described.

NOTES ON THE METALS GOLD, SILVER, AND ARSENIC IN THE COLLOID STATE.

BY T. CRAWFORD BOYD, F.R.C.S.I., D.P.H.,

CAPTAIN, I.M.S.

I. INTRODUCTION.

(*a*) Concept of the colloid state.
(*b*) Methods of preparation of colloids.
(*c*) Detection of colloid state and elementary analysis.

II. NOTES ON TOXICITY.

(*a*) Effects on Red Blood Cells.
(*b*) ,, ,, ,, Serum.
(*c*) ,, ,, ,, Mucous membranes.
(*d*) ,, ,, ,, Subcutaneous tissues.
(*e*) ,, ,, ,, Intravenous Injection.

III. EXPERIMENTS IN VITRO ON PARAMŒCIUM.

IV. EXPERIMENTS WITH COBRA VENOM.

INTRODUCTION.

The concept of the colloid state of matter has undergone many modifications since it was first described by Thomas Graham. To express this state nowadays, we may say that colloids "belong to the group of systems designated as polyphasic." By the term "phase is meant any homogenous part of a system differing from other parts of the system and separated from these by abrupt transitions" (Ostwald). To try and simplify this statement, we may take, for example, a diphasic system such as colloid gold: here we have a disperse phase consisting of a suspension of rigid gold particles varying from 0·1U in diameter to 1UU in size (Zsigmondy), separated by abrupt transitions from the other fluid separating phase, the continuous phase. According to whether the disperse phase is made up of rigid particles, or easily deformable particles, we have the two types of colloids—Suspensoid and Emulsoid, as an example of the latter class, gelatin may be taken.

In this short note it is only the suspensoids—Gold, Silver, and Arsenic—that will be dealt with.

Methods of Preparation.

Before proceeding to the actual preparation of the individual colloids, a word of caution is necessary concerning the glassware used; to be satisfactory this ought to be new and prepared carefully as follows:—

(1) Wash well in hot soap and water.
(2) Rinse out with tap water.
(3) Again wash well with dilute nitric acid.
(4) Get rid of (3) with frequent changes of tap water.
(5) Finally wash with freshly distilled water.

Unless the above steps are thoroughly carried out, success in the preparation of the colloids will not be attained,

DIAGRAM №I

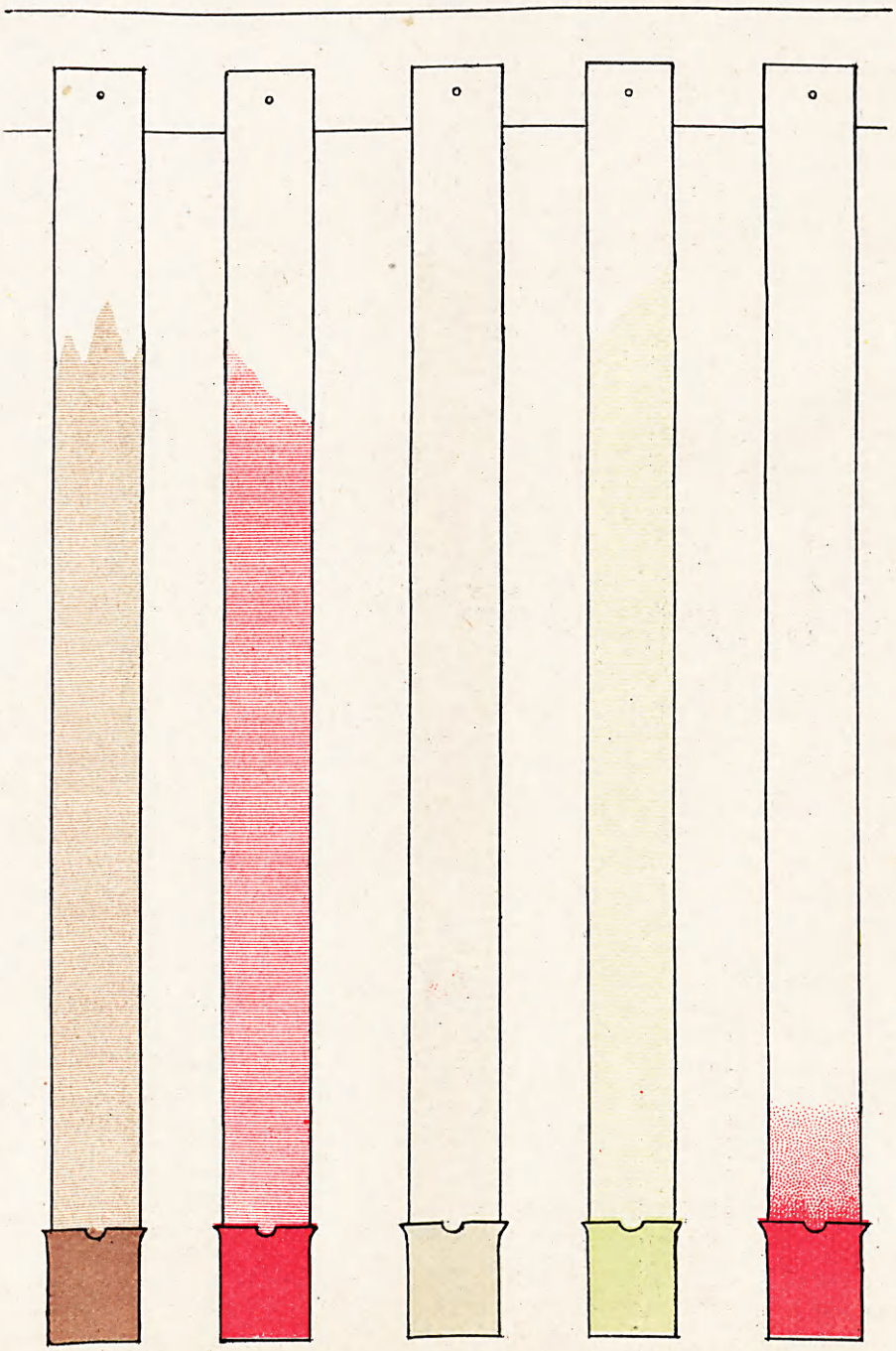

DIAGRAM № II

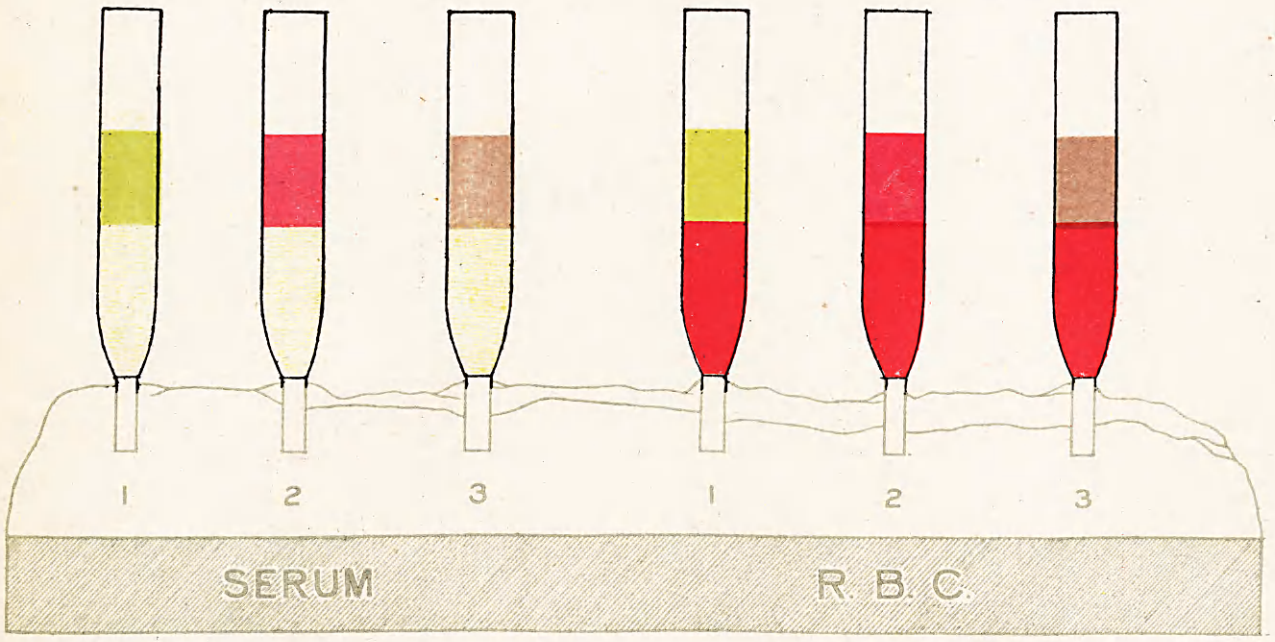

DIAGRAM Nº III (A)

DIAGRAM Nº III (B)

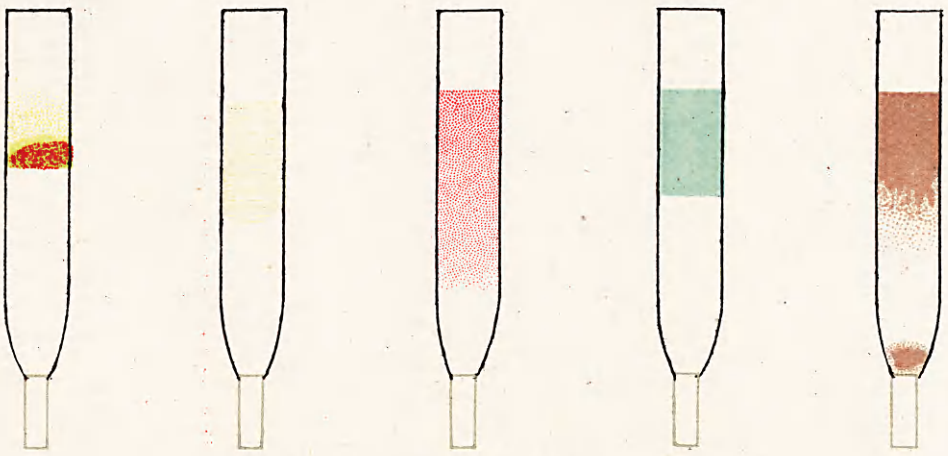

DIAGRAM Nº. (IV)

(A) COLLOIDAL GOLD.

According to the method used in the preparation of this colloid, two quite differently coloured suspensoids can be obtained—one of a blue colour, the other red.

Colloid Gold, Blue.—This suspensoid may be prepared by reducing a pure alkaline aqueous solution of gold chloride by means of a weak formalin solution. I believe the red colloid can also be obtained by this method, but I was unsuccessful and found it easier to prepare as follows.

Colloid Gold Red.—This is easily obtained by the reduction of pure gold chloride by means of tannic acid.

As soon as the colloid state is produced, a little sterile and carefully prepared gelatin is added. The object of this addition will be commented on later.

(B) COLLOIDAL SILVER.

The preparation of this is carried out by acting on the compound $AgNo_3$ $3NH_3$ with a weak solution of tannic acid, a blacky brown coloured suspensoid being obtained. A little gelatin is added as in (A).

(C) COLLOIDAL ARSENIC.

This can be prepared as a metallic sulphide by passing H_2S gas into a solution of AS_4O_6. After the production of the colloidal state the solution is boiled and gelatin solution is added.

Uses of Gelatin.—Pure suspensoids are very unstable and possess no keeping qualities, as rapid precipitation takes place. To overcome this difficulty, it is necessary to add small quantities of an emulsoid such as gelatin. These emulsoid particles are supposed to form a layer around the suspensoids and thus protect them from precipitation; from a practical point of view the method is certainly successful.

Tests carried out with the colloids prepared as above:—

(1) *Tyndall Phenomenon.*—To obtain this, the individual suspensoids were examined in a small glass vessel with parallel sides. (This piece of apparatus was kindly made for me by Professor Mukerji.) The source of light employed was an electric lamp fitted into a cup-shaped reflector. The whole lighting arrangement was then made light tight with black paper, except for a small hole situated in front of the centre of the reflector. In a dark room this apparatus gave a satisfactory pencil of light. Now, by allowing this pencil to enter the suspensoid in the glass vessel, a cone of light was obtained in all except the silver suspensoid, in which case it appeared as if the pencil of light was too weak to penetrate.

(2) *Diffusion Experiments.*—To test the diffusion of these suspensoids, a row of miniature test tubes were arranged as shown in diagram (1), and each filled to the height of about one inch with a solution of gelatin sufficiently strong to set to a firm jelly. To the tubes thus prepared were added in equal amounts Colloid Gold red, Colloid Gold blue, Colloid Silver, Colloid Arsenic, and as a control some aqueous solution of copper sulphate. After standing at bench temperature for two hours, the result shown in diagram (1) was seen, no diffusion in the case of the gold and arsenic suspensoids, a little want of definition at the junction line of the two liquids in the case of the silver colloid, and marked diffusion in the case of the copper sulphate. After 24 hours the $CuSO_4$ solution had almost reached the bottom of the test tube, whilst the silver showed only a little more diffuseness at the junction line; no attempt at diffusion was noted in the case of the other colloids.

(3) *Filter Paper Capillary Analysis.* (Method of Sahlbohm).—This method of analysis is an easy procedure to enable one to determine the sign of the electric charge carried by the suspensoid. The result obtained is given in diagram No. 2. The substances experimented on being the Colloids Silver, Gold red and blue; Arsenic and Congo red.

The Congo red was the only colloid that did not ascend, and from this we infer that it carries a plus charge on its particles, the other colloids tested carrying a negative charge.

(4) *Precipitation by Salts.*—Before the addition of any emulsoid to the above noted suspensoids, precipitation by salts was marked, after the addition of the emulsoid even in small quantities; stability on the addition of such electrolytes as NaCl and Mg_2SO_4 was greatly increased.

As a conclusion to this very imperfect introduction of a difficult subject, I must express my indebtness to the two following Authorities (Hatschek.—An introduction to the physics and chemistry of Colloids. Wolfgang Ostwald's Hand-book of colloid chemistry.)

II. NOTES ON TOXICITY.

In order to obtain some idea concerning the degree of toxicity of these suspensoids, the following experiments were carried out:—

I. *Experiment.* (Red Blood Cells)

A series of miniature test tubes were arranged in plasticine, and to each tube an equal volume of a 5 per cent. solution of washed goat's red blood cells was added, and finally an equal quantity of the suspensoids in the following order—arsenic, gold, silver. (*See* Diagram No. III B.)

Result.—Diffusion did not take place to any extent between the two solutions, and no hæmolysis was observed up to one hour.

II. *Experiment.* (Serum)

This was carried out on the same lines as experiment I, using serum in the place of the goat's red blood cells. (*See* Diagram No. III A.)

Result.—No precipitation was observed at the junction of the two liquids, nor did diffusion take place to any extent up to 24 hours.

III. *Experiment.* (Conjunctiva)

To ascertain the effects of the suspensoids on a delicate membrane, drops of the various suspensoids were instilled into the conjunctival membranes of a series of rabbits, only one eye was used in each animal, the other acting as a control.

Result.—No animal showed any sign of conjunctival irritation.

IV. *Experiment.* (Subcutaneous)

A series of rabbits were taken and each was injected into the flank as follows :—

Rabbit No. 1.—Twenty minims, colloidal gold.
Rabbit No. 2.—Ten minims, colloidal silver.
Rabbit No. 3.—Ten minims, colloidal arsenic.

Result.—None of these injections gave rise to any inflammatory trouble at the site of inoculation, nor was any systemic effect observed.

V. *Experiment.* (Intravenous)

No reactions in the foregoing experiments being obtained showing toxicity. A series of rabbits were given the following intravenous injections into the marginal vein of the ear.

Rabbit No. 1.—Ten minims, colloidal arsenic.
Rabbit No. 2.—Twenty minims, colloidal gold.
Rabbit No. 3.—Twenty minims, colloidal silver.
Rabbit No. 4.—Forty minims, colloidal gold.

These solutions were not rendered isotonic before injection.

Results.—In none of the series was any toxic symptoms noted.

CONCLUSIONS.

The above series of experiments seem to denote a very low degree of toxicity on the part of the suspensoid experiment ; in other words, their organotropic properties are very mild. So the next question that naturally arises is their, toxicity for the lower forms of animal life, or what may be called their parasitropic properties.

III. EXPERIMENTS IN VITRO ON PARAMÆCIUM.

Parasitotropic Properties.—To try and obtain some idea of this, experiments were conducted in vitro on paramæcium, using solutions of quinine hydrochloride and quinoidine as controls. The toxicity of the former drug has been worked out by Sir Leonard Rogers, I.M.S., and the latter by Major MacGilchrist, I.M.S. Antimony tartrate was also experimented with, as a metal in the non-colloidal state, and closely related to the metalloid arsenic, both chemically and pharmacologically speaking. The first series of experiments were carried out on a microscopic slide after the method used by MacGilchrist (*Indian Journal of Medical Research*, Vol. II, No. 1, p. 316).

EXPERIMENTS.

(A) One drop of paramæcium culture was placed on a slide and one drop of colloidal gold red was added. The result was observed under the microscope.

(B) One drop of culture and one drop of colloidal silver.

(C) One drop of culture and one drop of colloidal arsenic.

(D) One drop of culture and one drop of quinine hydrochloride, one per cent. solution.

Results.—(A) After ten minutes no difference could be noted in the behaviour of the paramæcium.

(B) Shortly after the addition of the silver the paramæcium congregated to the edge of the drop and violent movement was observed.

(C) The addition of the arsenic caused violent movement, and, shortly after, the great majority showed cessation of all movement.

(D) The quinine, on addition, caused violent movement with almost immediate cessation of all movement.

As the gold and silver suspensoids showed such litte toxicity, I proceeded only with colloidal arsenic, and instead of using the microscopic method, I used the method described by Sir Leonard Rogers (*B. M. J.*, Sept. 22nd, 1917, p. 383).

The following series of experiments show the results obtained by this method :—

EXPERIMENT.

Colloidal Arsenic.

Dilution.				24 hours.
1 in 20	with paramæcia	culture	...	–
1 in 40	„	„	...	+
1 in 80	„	„	...	+
1 in 160	„	„	...	+

Quinine Hydrochloride.

Dilution.		24 hours.
1 in 8,000	...	–
1 in 16,000	...	– after 5 minutes.
1 in 32,000	...	– + up to 45 minutes.
1 in 64,000	...	–

Antimony Tartrate.

Dilution.		15″	30″	24 hours.
1 in 2,000	...	+	+	–
1 in 4,000	...	+	+	+
1 in 8,000	...	+	+	+
1 in 16,000	...	+	+	+

+ = Living.
– = Died.

QUINOIDINE.

This was prepared from the commercial quinoidine as follows:—

The powdered drug was shaken up with dilute sulphuric acid and then filtered. The filtrate wa neutralised with calcium hydrate; this caused a precipitate calcium sulphate and quinoidine, and the latter re-dissolved by again acidifying with dilute acid. The solution was again filtered and evaporated down; this resulted in a dirty blacky green residue, amorphous in form, which rapidly took up moisture from the air. From this residue a one per cent. solution was made up by shaking violently with water.

Dilutions.	Medullary.	15″	30′	1′	24′
1 in 1,200 ...	+	+	–	–	–
1 in 1,600 ...	+	+	+	+	–
1 in 3,200 ...	+	+	+	+	–
1 in 6,400 ...	+	+	+	+	+

CONCLUSIONS.

From the above series of experiments we may conclude that the colloids, gold, silver, and arsenic, possess little toxicity for infusoria *in vitro* as compared with other drugs commonly used in protozoal infections of man. *In vivo* I am at present unable to state to what extent they are useful. In some cases of experimental surra infection in rabbits that were treated by injections of colloidal arsenic intravenously, the course of the disease seemed to be prolonged, but I have been unable to observe sufficient cases thus treated to draw any useful conclusions. In healthy young rabbits I have observed a slight leucocytosis twenty-four hours after injection (intravenous) of colloidal arsenic.

It may be of interest to note here a few experiments that I carried out on rats, which I injected with a lethal dose of cobra venom, and endeavoured to find out if the colloidal metals had any neutralising properties towards this venom. I was led to make these few experiments after reading Acton and Knowles' article in the *Indian Journal of Medical Research*, dated October, 1915, "Studies in the Treatment of Snake-bite."

EXPERIMENT (1).

In a series of miniature test-tubes a small quantity of a 12 sol. of cobra venom was placed and an equal quantity of the following solutions added and the result noted:—

(*a*) Colloidal Gold (red) + Venom Solution.
(*b*) Colloidal Arsenic + Venom Solution.
(*c*) Colloidal Silver + Cobra Venom.
(*d*) Gold Chloride Solution + Cobra Venom.

RESULTS.—(*See* Diagram IV.)

(*a*) No precipitate at first; slight precipitate appearing after some time.
(*b*) No precipitate.
(*c*) (No precipitate.) Precipitate after five minutes.
(*d*) Heavy precipitate.

From the above results I concluded that the only suspensoids likely to be beneficial would be silver and gold. To test the effects of these, a series of rats were taken and the following injection experiments carried out:—

EXPERIMENT (2).

0·1 gram Cobra Venom was dissolved in five cc. of water as standard Venom Solution.

10 minims of Venom Solution into Rat No. 1. Death in 6 minutes.
10 minims Venom + 10 minims C Gold into No. 2. Death in 8 minutes.
10 minims Venom + 20 minims C Gold into No. 3. Death in 20 minutes.
10 minims Venom + 10 minims C Gold; this was left in contact for 24 hours into No. 4. Death in 12 minutes.
4 minims Venom + 13 minims C Gold into No. 5. Death in 12 minutes.
4 minims Cobra Venom injected into No. 6. Death in 8 minutes.

Colloidal Silver.

5 minims of Venom injected into No. 7. Death in 10 minutes.
5 minims Venom + 5 minims C Silver into No. 8. Death in 15 minutes.
5 minims Venom + 15 minims C Silver into No. 9. Death in 18 minutes.
5 minims Venom + 15 minims C Silver left standing together overnight and injected into Rat No. 10. Death in 10 minutes.

CONCLUSIONS.

From these experiments it would appear that these suspensoids would have no beneficial effects in the treatment of cobra-bite.

A PRELIMINARY NOTE ON AN IMPROVED TECHNIQUE FOR THE DETECTION OF HOOKWORM EGGS.*

BY CLAYTON LANE, M.D.,
LIEUT.-COL., I.M.S.

IN the routine examination of stools for hookworm eggs the work of the Rockefeller International Health Board has proved that the centrifuge increases the positive findings by just over ten per cent. This has been proved independently by the workers in Trinidad and in British Guiana, their results being, curiously enough, identical within one place of decimals.

It is obvious on consideration that, should a portion of stool be completely suspended and then completely centrifuged, the amount of the deposit

* Received for publication, 25th March, 1918.