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TRANSACTIONS
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MEETING HELD AT THE MUSEUM ON THE 30th May 1902.

MAJOR F. J. CRAWFORD, I.M.S., IN THE CHAIR.

COLONEL MATTLAND exhibited two cases of oblique fracture of the shaft of the femur treated by extension alone without the use of splints. It was shown that the results were very good, only one-fourth of an inch of shortening occurring, as good a result, in fact, as can be obtained under any method of treatment.

CAPTAIN C. DOROVAN, I.M.S., read a paper entitled:—*Some recent methods of staining Malarial Parasites.*

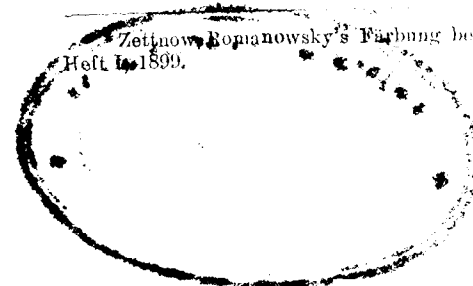
MR. PRESIDENT AND GENTLEMEN,—As I thought a description of some of the recent methods of staining malarial parasites in the blood, would be of interest to the members of the Branch, I have compiled the following from some recent books on the subject, with a few personal observations of my own. I am chiefly indebted to the works of Ziemann (1) and Argutinsky (2). It was in 1891, now more than ten years ago, that Romanowsky (3) discovered the property that solutions of eosine and methylene blue possessed when brought together in certain proportions, of forming a third or intermediate stain, having a particular affinity for staining nuclear substances. This third stain cannot be procured haphazard, but only after certain proportions of the two dyes are brought in contact.

It is to this dye thus obtained that we are indebted for the beautiful results in bringing out the details of the hæmamoebidæ.

Before giving the details of the two stains I intend especially to describe this evening, I consider it necessary to give a historical résumé of Romanowsky's stain and its modifications, culled from the work of Argutinsky above mentioned.

It was in July 1898 that Robert Koch found and subsequently of the same year, Zettnow,* that the addition of an alkali Lac

* Zettnow, Romanowsky's Färbung bei Bacterien, Zeitschr. f. Heft 1, 1899.

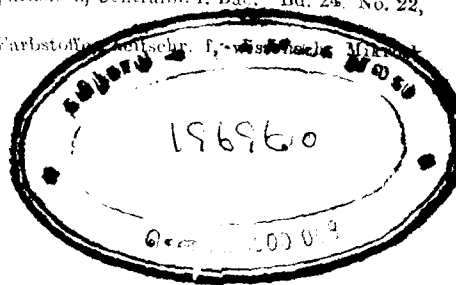


Potass. or Sodium) acted beneficially in Romanowsky staining. But the first publication which gave an explanation of the conditions underlying the production of this stain—the chromatin colouring substance derived from the methylene blue—was that of Nocht. Nocht* found in referring to Unna's† earlier investigation that the addition of an alkali to a solution of methylene blue is the surest method of procuring this derivative and consequently also for the success of the Romanowsky staining. Unna had in 1892, in a careful inquiry of the maturing of stains, sought to answer the question why by the use of old alkaline solutions of methylene blue, nuclear substances took on a decided red colour, while in freshly prepared neutral solutions of the same stain they were coloured purple. Unna was able to observe that in old alkaline solutions of methylene blue a red colouring material forms from methylene blue; he was able to extract by ether, this red colour substance, isolate and establish the fact that the red staining of the nuclei was brought about by this particular red derivative of methylene blue. After this investigation, Unna came to the conclusion that the source of this red dye was to be sought in the action of an alkali on methylene blue, and that it was not due to the influence of bacteria, oxidation or the action of carbon dioxide of the air. He finally found that the alkaline carbonates act better than the caustic alkalies. So Unna recommended the old (matured) alkaline solution of methylene blue "the polychrome methylene blue" used for the staining of protoplasm; however, a chromatin colouring by this derivative was not found by Unna. To these statements of Unna, made several years previously, are connected the interesting investigations of Nocht, concerning the chromatin colouring of the malarial parasites. After several ineffectual attempts to improve Romanowsky's special dye for parasites, by varying additions of a mixture of methylene blue and eosine, Nocht arrived at the result, that the dye is not due to the origin of a new colouring material in this mixture but that it is produced by the traces of another colour, which is contained as an impurity in the commercial methylene blue.

Guided by Romanowsky's observation, that only the very old solutions of methylene blue should be employed, Nocht remembered the procedure of Unna concerning the maturing of methylene blue, and used Unna's polychrome methylene blue, which contains in large quantities the impurities, the so-called methylene red and methylene violet. He found that on the least addition of Unna's polychrome methylene blue to Romanowsky's mixture, the chromatin colouring was considerably facilitated. Especially beautiful preparations were obtained by the addition of polychrome methylene blue which had been neutralised by dilute acetic acid. Consequently it came to the particularly interesting and important conclusion that Romanowsky's chromatin colouring of the malarial parasites is nothing else but staining with Unna's polychrome methylene blue and eosine.

Zur Färbung der Malaria-Parasiten, Centrallbl. f. Bac. Bd. 24, No. 22,

die Reifung der Farbstoffe, Zeitschr. f. mikrosk. Anat.



isolated by the evaporation of the chloroform. As Nocht did not consider this substance identical with either methylene red or methylene violet, he gave it the appropriate name of "Roth aus Methylenblau" or red from methylene blue. This is the substance, which with methylene blue and eosine gives rise to the Romanowsky staining. He found it in considerable quantities in all old alkaline solutions of methylene blue.

As its formation is considerably hastened at higher temperatures, Nocht placed the alkaline solution of methylene blue in a warm chamber at 50 to 60° C. for some days. For staining he took a 1% solution of methylene blue rendered alkaline by a 5% solution of sodium carbonate, which had been in the warm chamber from 1 to 2 days and added to it a 1% solution of eosine, until the colour of the latter had completely disappeared and then stained with this mixture from 5 to 10 minutes.

Ruge * stains with very dilute solutions of eosine and alkaline methylene blue. The latter he prepares either by heating for 48 hours a 1% solution to which has been added, on the first day, a 1% solution of sodium carbonate or by repeated heatings till steam rises. He then determines how much of a 1% solution of eosine, should be added to the alkaline solution of methylene blue for the formation of a precipitate and takes for the specific staining of the malarial parasites, the half (in some special cases, up to two-thirds) of this ascertained amount of eosine.

Before the addition of the 1% solution of eosine, he dilutes the alkaline solution of methylene blue to 2%. He stains by the intermittent heating of this eosine and alkaline methylene blue solution till steam rises, altogether for six minutes. For the demonstration of "Tüpfelung" in old preparations, the employment of less diluted solutions is necessary, about 1% or even 1/2%. Further, the more eosine is added to the alkaline solution of methylene blue, the longer is the staining required, for about a quarter of an hour; in this case, the preparation must be washed in alcohol.

Maurer † makes use of an alkaline solution of methylene blue (1% of methylene blue and 5% of sodium carbonate) and either heats it, according to Nocht for 2 days in a warm chamber, or allows it according to Zettnow, to stand at the room temperature, for 2 weeks before use; or as effectually places the mixture for 2 or 3 days exposed to the sun. He employs a 1% solution of Grüber's water soluble yellow eosine. He stains in diluted solutions; both the solutions of alkaline methylene blue and the eosine are diluted in 25 parts of water, equal parts of each are mixed together; thus 1/5% solutions of alkaline methylene blue and eosine are used. The staining lasts from half to one hour. After staining the preparation is simply

* Ruge, Ein Beitrag zur Chromatinfärbung der Malaria-Parasiten, Zeitschrift f. Bd. 33, Heft 2, 1900.
† Maurer, Die Tüpfelung der Wirtszelle des

subsequently, in a few minutes, a black precipitate. After 24 hours, filter and thoroughly wash with distilled water, the precipitate obtained, a greenish slimy mass. When washed two or three times, spread the filter paper containing the precipitate on a plate and place in the sun, until perfectly dried. Dissolve .5 gramme of the precipitate in 100c.c. of pure methyl alcohol. Merck's methyl alcohol for analytical purposes is the one used and recommended; when not procurable any other make of this pure alcohol will answer fairly satisfactorily but will necessitate a clearing up of the preparation. The ordinary methylated alcohol should not be used, as it contains impurities and is worthless. To stain, pour a sufficient quantity of the stain thus prepared on a slide containing a blood film, unfixed. Allow preparation to stain for five minutes, then wash with distilled water for half minute, till the film is of a light pink colour. As above mentioned, if the purest alcohol (Merck's) is not used, a clearing of the preparation will be necessary; for this purpose some absolute alcohol is poured over the slide for three or four seconds and then immediately washed off with distilled water. Dry the specimen, and examine with an oil immersion lens.

Leishman (5) has a modification of this method, in which he uses a 1% solution of methylene blue, made alkaline with .5% solution of sodium carbonate (matured by heat for two days) and .1% solution of eosine, B. A. extra quality. I have used this stain, but was not always satisfied with the results.

Staining properties.—The red cells are stained a pale pink, the protoplasm of the malarial parasites a light greyish blue and the nucleus (centrosoma, karyosoma, nucleolus or the so-called chromatin body) a bright dark pink, carmine violet or crimson lake.

As the leucocytes are especially well stained by this method and a fair index of the correct amount of staining, I may be pardoned in describing their appearances. The terminology is that of Ehrlich (6) and Grawitz (7); Ewing's (8) book may with advantage be consulted by members unacquainted with French or German—

(a) Polymorphonuclear, nucleus a beautiful pinkish-purple, compared to the Tyrian purple of the ancients; the cell

a very pale salmon pink, sometimes with a fine network and fine granulations stained dark pink.

(b) Mononuclear, nucleus of the aforementioned purple colour, cell a pale sky blue or eau-de-nil; at times, the cell protoplasm contains fine dots of an intense pink colour.

(c) Transition forms (formes de passage, uebergangsform), nucleus a pale greyish-purple, cell a light purplish-blue.

(d) Eosinophiles, nuclei pinkish-purple, the granules of the cell pink, varying in intensity from a dull pink to a bright reddishpink.

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of maturing the methylene blue. Make a 1% solution of Grüber's eosine, B.A. in distilled water. (Grüber's water soluble yellow and his pure eosine answer equally well.)

Dilute the 1% solution of alkaline methylene blue, as above matured and the 1% solution of the eosine, with 25 parts of distilled water. Keep these diluted solutions of methylene blue and eosine in separate glass-stoppered bottles.

To use.—A slide with the films fixed with alcohol and ether (I fix this for about two minutes, pouring a sufficient quantity of the fixing solution on the film and allow to dry at the room temperature: the results have always been good, and I see no reason for a prolonged fixation by alcohol and ether to bring out the details of the hæmamoebidæ) is placed in a glass trough, failing which the porcelain cover of a tooth brush dish or a dessert plate, on the slide is poured, simultaneously, equal portions of the diluted eosine and methylene blue solutions. The slide must be completely submerged in the stain. The trough is then tilted from side to side, to thoroughly mix the solutions. From 20 to 30 minutes suffices to bring about the desired staining. Remove the slide from the stain and wash in water, distilled preferable. Very often there is a uniform staining of the slide with a pink colour, this must be removed to give a clear preparation. To do this, pour some absolute alcohol over the slide and after a second or two, wash off the alcohol with water. Dry, mount in canada balsam, or without mounting, and examine with $\frac{1}{16}$ " oil immersion lens and a low eyepiece.

Results.—The parasites are stained a light blue, the nucleus, or more correctly the nucleolus, a brilliant dark pink. A peculiarity of this mode of staining (Romanowsky) is, that the red cells infected by tertian parasites (*Hæmamoeba vivax*) are dotted all over with pink spots, called "tüpfelung" in German, which word corresponds to our stippling. This dotting or stippling, is characteristic of tertian infection and of considerable diagnostic import. The dotting in the cases of malignant tertian is of a different character.

It has its disadvantages, in that it conceals the pigmentation of the parasite, a very minor detail. The red cells are stained a very pale yellowish-pink. The degenerations of the red cells are brought out very markedly, i.e., polychromasia and punctate

basophilia, thus giving in some cases of malarial infection a most unusual appearance to these cells. The leucocytes do not come out well, the staining is approximately the same as in Jenner, but there is not that definition of detail observed in the latter stain. The blood plates are very conspicuous, coloured a bright purple-pink, among the pale red cells.

Advantages.—The ease with which the very youngest forms of the parasites are detected, the "tüpfelung," diagnostic of tertian infection and the demonstration of degeneration in the red cells.

Disadvantages.—The want of permanency of colour

MEETING HELD AT THE MUSEUM ON THE 25th July 1902.

THE VICE-PRESIDENT (COL. W. R. BROWNE) IN THE CHAIR.

Captain T. H. SYMONS, I.M.S., showed a Mahomedan patient, aged 35, who had a swelling of the left foot with synovitis of the ankle and teno-synovitis of the dorsum of the foot. There was a history of injury twelve months before and one of syphilis many years ago. Considering all the circumstances Captain Symons thought the diagnosis lay between syphilitic disease and sarcoma, he inclined to the latter. The results of treatment would be watched.

Captain FAYRER, I.M.S., showed a case of heart disease in a student, aged 16 years, who had from infancy suffered from attacks of rheumatism, the last one an acute attack having been two years previously.

Captain FAYRER also showed a case of paralysis of the left arm and left side of the face in a man of 30 years who had a history of syphilis. The onset of the disease was gradual and aphasia occurred some time after the disease began. The patient was left-handed but could write with his right hand. During the patient's stay in hospital he had a fit in which there was loss of sensation as well as of movement in the effected parts, which up to then had shown some improvement.

Dr. M. KRISHNASWAMI AIYAR read a paper entitled *Epidemic Dropsy*.

Lieut.-Col. BROWNING said he was not disposed to accept the alleged Coimbatore outbreak, as freely as Dr. KRISHNASWAMI,

Dr. ROY was down there on famine duty, and there was one feature always present in the famine-stricken, œdema of the lower extremities. The one symptom of the cases they had heard of in Madras which was deserving of most consideration, was the fact of a number of people being attacked in one house or family.

C. B. RAMA RAU said that during his twenty years of work in Madras and Mofussil, he had not seen even a single case of dropsy. Dr. M. KRISHNASWAMI AIYAR was lucky in

alighting on so rare a disease while yet so young, and he would have placed the members of the profession under a great obligation if he had shown them some of his cases.

It was rather peculiar that though epidemic dropsy prevailed in Madras both this year and the last, it should have been confined to a few families and to the practice of one or two members only.

Lt.-Col. MAITLAND,—A few days after Dr

had been seen by other members, and it would be interesting to hear what was thought of them. He did not wish to deny the possibility of the existence of epidemic dropsy as a definite entity, nor its presence in Madras, but he was desirous before recognising it of being sure that all other causes of oedema had been excluded by the most thorough clinical examination, since the few cases he had up till then seen did not carry absolute conviction to his mind.

Major CRAWFORD had cases under his care in the General Hospital and they were of the type described by Dr. KRISHNASWAMY. One of them was a European who had resided for many years in the town of Madras.

The Chairman said he had had much experience of dropsy occurring in the course of malarial cachexia and he would like to hear if these cases had been examined for malarial parasite. He felt that only on the strongest evidence should a presumably new disease be admitted to the nomenclature presenting no characteristic symptoms or pathology. Apart from the evidence of infectiousness he was inclined to await further investigation, clinical and microscopical, before being able in his own mind to exclude the many known causes of dropsy in this country. He did not think sufficient stress was laid on these points by the contributors to the discussion.

EPIDEMIC DROPSY.

INTRODUCTORY.

It has been my fortune during the short period of my private practice at Madras to see a few cases of Epidemic Dropsy. It is a very rare epidemic and one which has been observed by only a few. It has been described by these few under various names. It was by very many confounded with Beri-Beri when it first broke out in Calcutta in 1877. Owing to the absence of any information on the subject of this disease in many text-books of medicine, the want of knowledge in most of the practitioners about the existence of such a disease, the fact that only a few people resort to English and medical men, owing to all these causes, a good many cases went unnoticed at the beginning and it was only when the disease was common that it was recognised both here and at Calcutta in 1901.

Several contributions having reference to this disease have appeared in the *Indian Medical Gazette* for the years 1877, 1878, 1879, 1880, 1881, 1882, 1893, 1894, the authors being Drs. O'Brien, Cayley, McConnell, Crombie, Maurice Smith, Alexander Nairne, Rammay Roy, D. B. Smith, Chambers, Harvey, Kasthighir, Tameez Khan, Shirley Deakin, and K. McLeod. I have appended a full list of the literature on the subject. Owing to the scattered nature of information on this disease, I have attempted in this paper to bring together all the available information on this subject together with my own experience.

I shall now preface my paper with a definition of the disease as given by Dr. K. McLeod with slight modification.

the August issue of the *Indian Medical Gazette* about the re-appearance of this disease in Calcutta. When I spoke to some of the other medical practitioners of this place about the advent of this rare epidemic to Madras, they said that they had seen a large number of cases of dropsy with fever and that they were treating these cases as malarial. I must here before proceeding further, thank Capt. J. W. Cornwall, I.M.S. (then Health Officer) for his kind help enabling me to see some more cases here and also for getting down some information about the disease in Calcutta. These cases which were not mine and which were simply shown to me for diagnosis were all complicated with malarial fever and hence were mistaken to be malarial fever with dropsy as a sequela and were treated as such.

Unlike the previous epidemics of this disease between the years 1877—1880 which broke out during the cold weather to disappear during the hot weather and the rains, the last epidemic of this disease (1901) both here and at Calcutta broke out during the hot weather and rains to disappear with the onset of the cold weather. A few cases have also appeared during the hot weather of 1902 to disappear in the cold weather. Prior to the epidemic of 1901 it was thought that there was much close relation between this disease and the cold season, and that the disease could not exist in the hot weather so much so that when the epidemic broke out in the hot weather in 1901 it was feared that we might have a severe recrudescence of it in the cold weather. But strangely enough the disease subsided in the cold weather of 1901 to reappear in the hot weather of 1902. We have, therefore, to suppose that the disease may break out any part of the year without reference to season.

ÆTIOLOGY.—We shall now divide the causes into predisposing and exciting or determining causes and take them separately.

Predisposing Causes :—

1. *Race* :—It is very remarkable that the disease prevailed in all the localities it visited mostly among persons of a particular race. In Calcutta natives were by far the greatest sufferers. A few Eurasians and Armenians were also affected. Among Natives, Mahomedans are said to have suffered more severely and frequently than the Hindus. Europeans have been entirely exempt. Dr. Crombie writing about Dacca states that the disease attacked

all classes of society except Europeans. At Shillong, Bengalees and Assamese were mostly affected, Khasias rarely and Europeans never. In South Sylhet the disease was confined entirely to the Indian coolies working in tea gardens. In the Mauritius it was almost entirely confined to the Indian population, especially in the country districts: among the Indians it was remarked that those from Calcutta were more liable to be affected than those from Madras. Europeans were entirely exempt.

In Madras all the cases that I saw were among high class Hindus

