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ON THE OCCURRENCE OF OEDEMA AMONGST CHOLERA CONVALESCENTS.

by

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With the permission of the Director of Medical Services, Hong Kong, and through the kindness of Dr. Shaw and later of Dr. Wilkinson, the members of the Department of Physiology of the Hong Kong University were given the opportunity of studying some aspects of the problems that confronted the Government Medical Officers during the cholera epidemic of last year.

During this epidemic, observations both clinical and biochemical were made on about 200 patients during acute and convalescent stages, and latterly we were fortunate enough to be able to make repeated blood investigations on the same patients at successive stages of the disease.

As we present these results we are extremely conscious of a feeling of disappointment that certain obvious lines of investigation were not pursued. By way of explanation rather than excuse, we would at the beginning therefore make the following observations. In an emergency of such a type and size as this epidemic—there were 1,320 cases with 742 deaths between the 1st of August and 30th of September—we were confronted with problems for which we had not a carefully though out scheme of action prepared.

The only references we had to detailed and extensive biochemical investigations on cholera cases were those of Schmidt made as long ago as 1850 and Sellards* in the Philippine Isles in 1911, but as neither of these papers was available in Hong Kong most of our labour was essentially of the ground-work type and it was not until towards the end of the epidemic that we were able to follow a scheme which

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Through the kindness of the Bureau of Science, Manila, P.I., we have since obtained a copy of this latter article.

had the value of experience behind it. On account of lack of apparatus we were unable to carry out two most important determinations those of plasma proteins and alkali reserve of the blood. Concerning methods, we found it necessary to make so many different determinations on one sample of blood that it became necessary to work out modifications of established methods to enable this to be done. For example it was found necessary for two of us (K.D.L. & S.Y.C.) to devise methods of estimating non-protein nitrogen and blood urea on 0.2 ccs of blood. By means of these methods and carefully planned team work we were, towards the end of the epidemic, able to estimate the following on about 5 ccs. of blood:—pH, plasma and whole blood chlorides, urea, non-protein nitrogen, pyruvic acid, glucose, calcium, phosphorus, red cell and white cell volumes and plasma volumes. The ultimate value of these observations is rather lessened by the fact that histories in most cases were not available. In some instances we kept brief case notes ourselves and these will be referred to later.

As far as one can gather from reports of epidemics in other parts, the patients were kept in hospital in Hong Kong for a much longer period than is usually possible; they were not discharged as soon as the acute stage was over, but were kept in hospital until two rectal swabs were vibrio negative, and it was to those cases during this convalescent period that our attention was first drawn. Dr. Shaw had noticed that many of these cases developed a very marked oedema. As a result of the extreme emaciation and dehydration of the acute stage, the oedema of the convalescent was readily recognised and presented striking characteristics. The general picture of the convalescent patient was somewhat as follows:—the muscles were flabby, extremely atonic, greatly reduced in size, and devoid of almost all tissue fluid; there was hardly any subcutaneous tissue and the skin itself was dry and almost of paper thickness and had lost its elasticity and resilience. On pinching, the skin would remain creased for a long time and the imprint of the bell of a stethoscope lightly applied even over the forehead would remain unobliterated for some minutes; this imprinting was readily distinguishable from the pitting of oedema, (although at first we made the mistake of thinking it was oedema of the true skin) the dry skin giving a clear-cut imprint, the oedema leaving a less sharply outlined pit rather than an imprint. To the feel, the superficial tissues were more jelly like than is usual in anasarca of cardiac origin and pitting impressions filled in very readily. In those cases well and strong enough to adopt their usual squatting position the commonest site of the oedema was the dorsum of the foot, sometimes extending part of the way up the leg; the upper limits of the fluid were generally clear cut and easily recognisable; in more severe cases the thighs were also involved, in a few the scrotum was distended and the prepuce oedematous, while in one or two there was marked ascites; some of these more severe cases also showed oedema of the hands. In those cases who were still too

weak to squat, the oedema extended along the posterior aspect of the thighs, buttocks and back.

Another and smaller group showed oedema that differed from the above in two important aspects of type and distribution. The oedema was much less fluid, the pitting more permanent, the skin itself was stretched and glossy, and the distribution was general, involving the limbs, abdominal wall and face but without ascites. In two cases the face was so markedly affected that the eyes could hardly be opened.

Onset and Termination of Oedema.

As explained above, accurate histories were not kept and in the absence of dates of the onset of the disease in each case, we are not able to give the exact time relation of the onset of the oedema. The only relatively fixed points we have in each case are the dates of admission to hospital and of the transference to the convalescent wards. After the acute stage the patients were transferred to a convalescent ward and in many cases we noted these dates, but there is no certainty that the date of transference coincided with the end of the acute stage. The date of appearance of oedema, was accurately noted. Using therefore the date of admission and that of transference to the convalescent wards as rough guides to the period of the acute stage, we have the following data concerning the onset of oedema.

Of 38 of these cases the average period between admission and transference to the convalescent wards was 6 days; the average period between admission and onset of the oedema was 9 days with a range of from 3 to 23 days, so that on an average the oedema appeared about 3 days after the acute stage terminated but in a number of cases the oedema was markedly delayed, not appearing till between 2 and 3 weeks after the disappearance of all acute symptoms. Some of these cases were kept in hospital for investigation, others awaiting negative rectal swabs, which facts explain our high incidence of oedema.

The large group with the fluid type of oedema all made an uneventful and spontaneous recovery, the average number of days duration for 17 men was 22.5 ± 1.26. The men in one ward were put on an increased protein diet and many of these patients showed rapid improvement, the additional diet consisted of 12 ozs of rice and ⅔ lbs. of meat per day per patient, representing approximately an additional 40 grams of protien per day.

Oedema amongst the smaller group did not show any signs of spontaneous improvement and will be considered separately. The following considerations apply to the large group only.

Sex Incidence of Oedema.

Sex frequencies sufficient to give reliable ratios were not kept but there is no doubt that oedema was much more common amongst men; this was one of the first characters of the oedema noticed by Dr. Shaw and the nursing staff, and the following figures indicate the degree of sex difference. Of the 38 oedematous cases cited above, 32 were males and 6 females, and of another group, none of which developed oedema, 9 were males and 19 females. The average length of stay in hospital of this latter group was 21 days and since the average period of onset after admission was 9 days it can be taken that these non-oedematous cases were unlikely to develop oedema from a similar cause after discharge from hospital.

Technique of the Various Investigations.

Before beginning our discussion of the possible causes of this oedema we shall first briefly indicate the methods used in our laboratory investigations.

The blood was taken from the median basilic vein under the usual sterile precautions and then delivered immediately into a number of small labelled test-tubes as follows:—the first for pH and plasma chlorides, contained paraffin and the blood was passed under the oil to prevent loss of CO_2; the second for the estimaton of pyruvic acid contained weighed trichloracetic acid solution; the third for urea, non-protein nitrogen and glucose contained potassium oxalate as anti-coagulant; the fourth for calcium and phosphorus estimations contained no anti-coagulant; the rest of the blood was passed into the fifth tube which contained ammonium and potassium oxalate and this blood was used for sedimentation rates. In all about 5 ccs. of blood was drawn for each set of determinations.

The pH was determined as soon as possible after withdrawal by means of Cullen's (1929) quinhydrone electrode method. Plasma and whole blood chlorides were estimated by Patterson's (1928) micro-modification of the open Carius method using 0.2 ccs. of whole blood or plasma, while the cell chlorides were calculated from these results and the plasma volume. The sedimentation rates were determined by the automatic photographic method described by Ride (1936) and Ride *et al* (1936) using Wintrobe (1930) tubes, the cell and plasma volumes being determined by the method of Millar (1925).

Urea was measured on 0.2 ccs. of blood using Ling & Cheng's (1937) modification of Archer's micro-method, and on another 0.2 ccs. of blood the non-protein nitrogen was determined by Ling & Cheng's (1937a) modification of Folin's micro-method.

The method of Haggedorn & Jensen (1932) was used for glucose and Peters & Thompson's (1934) modification of the Neuberg-Case method was used for pyruvic acid determinations.

The colorimetric determinations for urea, non-protein nitrogen, pyruvic acid, plasma proteins and phosphorus were made by means of a densitometer obtained from the Cambridge Instrument Co.

Plasma proteins were estimated by Greenburg's (1929) modification of Wu & Ling's colorimetric method.

Cause of Oedema.

Where the total quantity of body fluids is grossly changed, we may expect a corresponding change in the interstitial fluids; a diminition is of course common in the acute stages of cholera and an increase in both occurs also in the acute stage where large quantities of saline have been given intravenously. These conditions do not appertain in the convalescent stage and hence we shall not discuss them further.

In general it may be stated that where the total body fluids are within normal limits, the constancy of the interstitial fluids is maintained by a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic pressure differences between the fluids in the tissues and in the capillaries,—mainly due to the plasma proteins and salts. Other factors which influence this balance are the permeability of the capillary walls and the elasticity or resilience of the surrounding tissues. In so far as renal function may affect the protein and salt content of the blood and blood volume, it also has a very important effect on tissue fluid formation and circulation.

The hydrostatic pressure in the capillaries is difficult to measure and our only method of making some estimation of it is by means of the arterial and venous blood pressures and of the cardiac output. In our work we took the arterial blood pressure and pulse rates only, and in 39 oedematous and 26 non-oedematous male adult convalescents we obtained the following results:

TABLE I.

Oedematous			Non-oedematous		
No.	B.P.	Pulse	No.	B.P.	Pulse
39	115/74 mm. Hg.	71	26	118/72 mm. Hg.	72

Statistically these differences are not significant. In none of these cases could any signs of heart failure be demonstrated and with the reservation that the emaciated condition made it extremely difficult to percuss cardiac dullness—in the supine position the heart fell right away from the thoracic wall—no cardiac enlargement could be detected. From this it seems reasonable to argue that increased capillary hydrostatic pressure was not responsible for the oedema.

The two most common factors which affect the permeability of the capillary wall are oxygen-lack and toxaemia. Concerning the presence of neither of these have we any evidence at all *during convalescence*. During the acute stage however, it is a different story; cyanosis and capillary stagnation point to definite tissue oxygen-lack;

experimental evidence has established the presence of both endo- and exo-toxins in cholera (Rogers 1921), but in our opinion the toxic effect is more sudden and of shorter duration than that of the anhydraemia. Evidence of the mildness and temporary effect of the toxaemia is given by the remarkable way in which patients recover both their normal appearance and outlook when dehydration stops or is relieved, and also by the way patients frequently show a very temporary thermal response to intravenous infusions, which response is most probably explained by an increased absorption of the toxins from the gut due to the re-establishment of an efficient circulation through the intestines. This temperature spike is of a comparatively short duration and does not extend into the convalescent period at all. These facts seem to suggest that the effect of toxins on the capillary wall during convalescence is negligible and all the more unlikely as there are no other evidences of their activity during this period. We shall refer to the action of toxins in the acute stages in a later paper.

Concerning the elasticity of the skin and subcutaneous tissues, we have already remarked on its almost complete absence and although this condition was not likely to be the cause of the oedema it almost certainly played a passive part in favouring the accumulation of excess tissue fluid—an accessory after the fact as it were.

The role of the kidneys we may consider from evidence set out under two headings, urinary and blood analyses.

Urine Analyses.

Certain of the urine analyses are best shown in tabular form.

TABLE II.

Cases	Number	Reaction	Sugar	Albumin
Oedematous	27	Acid 25 Neut. 1 Alk. 1	—ve 25 Trace 2	all negative
Non-oedematous ...	32	Acid 20 Neut. 3 Alk. 9	—ve 31 Trace 1	all negative
Post-oedematous ...	18	Acid 17 Alk. 1	—ve 18	all negative

Urine analyses of 77 convalescent cholera cases showing the reaction, glucose and albumin tests of cases with and without oedema and cases that had recovered from oedema.

The remarkable thing about these urines was the comparative absence of abnormalities; apart from the trace of sugar in the three cases recorded in Table II, and acetone bodies in one case, there were no other abnormal substances detected except indican; we found indican in three convalescent cases, but we are unable to indicate the exact frequency of indicanuria in either our acute or convalescent cases, because its presence was not investigated as a routine. Our attention was drawn to its presence just before the end of the epidemic when a sample of urine that had stood for a day or two in the laboratory was noticed to have undergone a colour change. This was proved to be due to indican, and we then found indicanuria in a number of the remaining acute cases, the significance of which will be discussed later.

The results of the examination of specific gravity, urinary urea and chlorides are set out in Table III.

TABLE III.

GROUP	SPECIFIC GRAVITY		UREA		CHLORIDES	
	No. of Cases	Mean Values	No. of Cases	Mean Values	No. of Cases	Mean Values
Oedematous	27	1010.3 ± 0.40	46	0.586 ± 0.048	43	0.498 ± 0.048
Non-oedematous ...	32	1012 ± 0.69	40	0.733 ± 0.076	50	0.524 ± 0.043
Post-oedematous ...	18	1009 ± 0.34	18	0.433 ± 0.037	12	0.133 ± 0.045

The mean specific gravity, urea and chloride content of 105 cholera convalescent cases are here set out in three groups, oedematous, non-oedematous and post-oedematous. The urea is expressed in grms per 100 ccs. of urine and the chlorides as grams of NaCl per 100 ccs. of urine.

The differences between the means of these groups were estimated and are set out in Table IV together with remarks as to their significance; in order to make the comparison as strict as possible we have chosen three times the standard error as our criterion of significance throughout this paper.

In considering first the specific gravity, the figures given have been corrected to 15°C; they are undoubtedly on the lower borders of the normal range, and the only differences established are that the post-oedematous urines are on the average more dilute than the non-oedematous ones. This lowered specific gravity may be due to greater concentrating powers of the non-oedematous kidneys or may be simply an expression of the increased urinary excretion during the loss of oedema fluids, but in the absence of 24 hour specimens and their analyses, we cannot reach a final conclusion as to the significance of this finding.

TABLE IV.

GROUP COMPARED	SPECIFIC GRAVITY		UREA		CHLORIDES	
	Differences in Means	Remarks	Differences in Means	Remarks	Differences in Means	Remarks
Oedematous and Non-oedematous ...	1.8 ± 0.80	N. S.	0.147 ± 0.085	N. S.	0.026 ± 0.053	N. S.
Oedematous and Post-oedematous ...	0.6 ± 0.53	N. S.	0.153 ± 0.053	Sig.	0.365 ± 0.066	Sig.
Non-oedematous & Post-oedematous ...	2.4 ± 0.78	Sig.	0.300 ± 0.085	Sig.	0.391 ± 0.062	Sig.

The mean values of the specific gravity, urea and chloride content of the urines of cholera convalescents given in Table III are here compared and the differences between the groups set out and their significance noted. N.S. = not significant, Sig. = significant.

It should be noted however that the specific gravity figures indicate no difference in the concentrating powers of the kidney between the oedematous and non-oedematous groups of convalescents, and the absence of albumin in all three groups of cases is further evidence of the absence of the effect of toxins in the convalescent period.

Urinary Nitrogen.

Our nitrogen excretion investigations were at first confined to urinary urea and ammonia, but later for various reasons urea and total nitrogen were estimated instead. Peters & Van Slyke (1931) state that urea ordinarily constitutes 80-90 per cent. of the urinary nitrogen but they quote Folin's (1917) work in which he showed how this percentage may be lowered to 60 by merely lowering the protein intake, which figure must be taken as normal for persons on low protein diets such as our convalescents. Such comparisons to be of any real value should not only therefore take the protein intake into account but should be carried out on 24-hour urine samples. This unfortunately we did not do. In acute cases, especially females, this would be very difficult to do thoroughly, but in convalescents it could have been attempted had we realised its importance early enough. We therefore have to present data from single urinary samples taken generally about 4 hours after the morning meal, being concious at the same time of the limitations of its usefulness. There is however this to be said in its favour; it is comparative data of groups of cases to each of which the same objection applies, and we submit that as long as we consider the comparative and not the absolute values of the data from the groups our conclusions should be valid.

For the purposes of this comparison the urea nitrogen and total nitrogen were measured for each, and expressed as gms. per litre and then the ratio of the former to the latter was estimated. In all, 35 cases were thus examined, 14 being oedematous, 10 non-oedematous and 11 having recovered from oedema. In the first group the mean ratio was .56±.04 in the second .75±.06 and in the third .56±.03. None of these groups was significantly different on this score from the others.

The main function of urinary ammonia, although depending in amount on the acid-base balance of the blood, is not to preserve this balance, but rather to conserve the fixed base of the blood. Assuming that the total urinary nitrogen is mainly urea and ammonia nitrogen, a fall in the urea-nitrogen/total-nitrogen ratio must indicate two things, (a) the need of the body to conserve its fixed base and (b) the ability of the kidney to carry out one of its important functions, namely, that of converting urea into ammonia.

Applying this argument to the data given above, we see that the low ratio indicates that during convalescence the body is conserving its fixed base, is still as it were, paying the price of its gross fixed-base loss during the acute stage.

Concerning the kidney function of converting urea into ammonia, we are entitled to conclude from these data that this function is intact, only if the kidney is the sole site of ammonia formation; with increased protein katabolism we might expect to find increased ammonia output depending on increased deaminization, but since during convalescence there is no other evidence of increased protein break-down we can consider that an increase in urinary ammonia means an efficient renal transformation of urea into ammonia.

Whatever be the value of these arguments concerning kidney function there is one fact that stands out in these figures and that is that they point to no difference in kidney function in the oedematous and non-oedematous cases.

Turning now from these nitrogen ratios to the urea figures themselves, we find, as we might expect from the specific gravity figures, that the urea concentration is low in all cases, while on comparing the three groups as before, we see that again the oedematous and non-oedematous ones show no differences, whereas the post-oedematous group shows urea concentrations lower than both the other two. The chloride figures show these similarities and differences in an even more striking manner, the post-oedematous urines being much lower in chloride content than the other two groups between which no difference is established.

Summary of Urinary Analyses.

We have given statistical data concerning the urinary analyses of three groups of cholera convalescents, those without and those with

oedema, and those in whom all trace of oedema has disappeared. When considering reaction, specific gravity, urea content, urea-nitrogen/total-nitrogen ratio and chloride content of the urines, in none of these characters does the oedematous group show any differences from the non-oedematous group, although in many cases the post-oedematous group shows a marked difference from the others, always in the direction of a lower concentration. If the corresponding blood analyses of these groups show the same similarities, we can assume that kidney function is similar in both the oedematous and non-oedematous cases and hence the kidney cannot be held primarily responsible for the one difference between them, the presence of oedema. We shall therefore now turn our attention to the blood analyses in convalescent cases.

Blood Analyses.

The blood urea must obviously vary directly with its rate of formation and inversely with its rate of elimination by the kidneys and conversion into ammonia. Its rate of formation must depend on the rate of protein katabolism; an increase in the latter may be brought about by increased protein intake or increased body protein destruction. In the acute stage of cholera, absorption from the gut of products of protein digestion can be taken as nil, and the diet during convalescence was definitely deficient in protein until it was augmented as previously described; we can take it therefore that protein intake during both the acute and convalescent stages was thus not increased; and any rise in blood urea must therefore be due to increased body protein destruction or decreased urea disposal. In the absence of urea clearance data, the blood urea figures do not allow of deductions being drawn concerning kidney function in every case. High blood urea, where there is greatly increased protein breakdown, is still compatible with unimpaired, though insufficient renal urea disposal. A blood urea within average limits does, under these circumstances of protein intake, indicate efficient—even though it may be impaired—kidney function.

The blood urea figures of 45 convalescents, where protein intake was low and protein katabolism certainly not increased, were all within normal limits.

A reference to Table V shows that the same statement applies to the whole blood chlorides and non-protein nitrogen, and furthermore when the values in groups are compared, in no case are the differences found to be significant. We are led to the conclusion therefore that the kidneys in these two groups, by dealing with similar bloods and producing similar urines, do not display any differences in function, and hence it is unlikely that the presence of oedema, which is the outstanding difference between these groups, is renal in origin.

TABLE V.

GROUPS OF CASES	BLOOD UREA		BLOOD N.P.N.		WHOLE BLOOD CHLORIDES	
	No. of Cases	Mean Values	No. of Cases	Mean Values	No. of Cases	Mean Values
Oedematous	26	22.3 ± 1.71	8	43.6 ± 5.97	14	475.7 ± 11.91
Non-oedematous ...	25	24.2 ± 1.55	14	35.1 ± 3.04	20	468.5 ± 15.35
Differences between Group means	1.9 ± 2.3 (not significant)		8.5 ± 6.7 (not significant)		7.2 ± 19.4 (not significant)	

Mean values of whole blood chlorides, urea and non-protein nitrogen in oedematous and non-oedematous convalescent cholera patients. The chlorides are expressed as gms. of NaCl, urea as mgms. and N.P.N., as gms. per 100 ccs. of whole blood.

In the absence of detailed clinical notes it is difficult to assess the part played by avitaminosis B_1 in this larger group of oedema cases but the pyruvic acid values are worth considering. In 35 non-oedematous convalescents the mean pyruvic acid content of the blood was $.703 \pm .056$ mgs. %, while that of 14 oedematous convalescents was $1.671 \pm .162$, giving a difference of $.968 \pm .172$, which is highly significant. The oedema cases thus on the average had a definitely higher blood pyruvic acid content than the non-oedematous cases, and hence avitaminosis must be considered as a contributing factor to the prevalence of oedema amongst these cases.

TABLE VI.

BLOOD PYRUVIC ACID CONTENT	
Before Betaxin	After Betaxin
1.94 mgms. per 100 gms. blood	0.90 mgms. per 100 gms. blood
1.02 ,,	0.26 ,,
2.19 ,,	1.07 ,,
1.80 ,,	0.68 ,,
0.76 ,,	0.58 ,,
1.01 ,,	0.22 ,,

Showing the blood pyruvic acid content of six oedematous convalescent cases, before and after administration of Betaxin.

In some of these cases a therapeutic test was applied, vitamin B_1 being administered by means of intra-venous injection of 5 mgms. of Betaxin and the effect on the pyruvic acid is shown in Table VI.

In all these cases except the last, the fall in the pyruvic acid was accompanied by a marked lessening of the oedema; the other oedematous cases were not put to this therapeutic test, but these figures given are sufficient to substantiate the statement that avitaminosis B_1 was in some cases a contributory cause to the oedema.

Returning to our original argument, we are now left with changes in osmotic pressure as the last possible cause of the oedema; plasma protein determination would have settled this point, but these we were not able to do until almost at the end of the epidemic. We were however able to obtain important indirect evidence on this point; we know that the sedimentation rate of blood depends amongst other things on the plasma proteins. We estimated these rates in 17 samples of convalescent bloods and found the range of the rate was from 14.4–63.0 m.m., per hour with an average for 8 males of 36.13 ± 3.54 m.m., and for 9 females of 46.33 ± 4.45 m.m.

These rapid rates are compatible with low plasma protein contents, and would undoubtedly interfere with the reabsorption of tissue fluid into the venous end of the capillaries, an explanation which has been put forward in discussing the occurrence of nutritional oedema both in middle Europe and in North China.

Clinical Histories.

We give here some brief clinical notes of two cases typical of this type of oedema in order that a comparison may be made between them and the other oedema group to be discussed presently.

Case No. 711, male 59, hawker, admitted 28.8.37, oedema appeared first on 4.9.37. Moved to convalescent ward on 6.9.37. 18.9.37, marked oedema of both feet and legs; very fluid in type making movements of legs and ankles cumbersome. B.P. 96/72, pulse, 64.

23.9.37 blood sugar 79 mgms. %, blood urea, 24 mgms. %.

25.9.37. Oedema had spread up the posterior aspects of both thighs and was present in the scrotum and prepuce. Interdigital spaces of the hands were also oedematous. Muscles not tender and power good; patient able to lift both legs off the bed without flexion of knees. Knee and ankle jerks absent on both sides, biceps jerks present on both sides. Mentality dull.

28.9.37. Oedema of scrotum and prepuce decreased, but still present on posterior aspect of thighs, and still marked on dorsum of foot, ankle and half way up the legs.

No tenderness in calf muscles and muscle movement good except insofar as it is impaired by the large excess of oedema fluid. Arterial sounds not heard except over femoral arteries on deep pressure.

Right knee jerk positive, otherwise no change in tendon reflexes.

29.9.37. Oedema still marked on dorsum of feet. Blood sugar 114 mgms. %; blood urea 17 mgms. %; pyruvic acid 2.15 mgms. %.

1.10.37. Oedema of scrotum, penis, thighs and legs completely disappeared; slight oedema on dorsum of feet only. Arterial sounds not heard over any artery; left knee-jerk positive.

Patient made an uneventful recovery and was discharged free of oedema on 13.10.37.

Case No. 1048, male 56, coolie, street-sleeper, admitted 12.9.37; transferred to convalescent ward 15.9.37.

19.9.37. B.P., 126/84, pulse 78.

24.9.37. Oedema appeared on dorsum of feet.

29.9.37. Oedema had involved ankles, legs, posterior aspect of thigh and scrotum. Ascites present. No muscle tenderness, muscle power good; able to walk and to lift both legs off bed without flexion of knees. Heart not enlarged and sounds normal. B.P., 130/70, pulse 70. Arterial sounds heard over femoral arteries and right dorsalis pedis only. Knee, ankle and biceps jerks and abdominal reflexes present and equal on both sides. Blood sugar 126 mgms. %; blood urea, 10 mgms. %; N.P.N., 22 mgms. %; pyruvic acid, 1.95 mgms. %.

1.10.37. Oedema reduced in severity still marked on ankles, feet and legs; skin loose and wrinkled. Tendon reflexes still positive.

5.10.37. Blood sugar 111 mgms. %; blood urea, 11 mgms. %; pyruvic acid, .93 mgms. %. Oedema now very slight. Patient made uneventful recovery and was discharged on 27.10.37 oedema free.

Conclusions Concerning the First Type of Oedema.

There can be no doubt that members of the poorest class of Hong Kong Chinese are habitually in a state of malnutrition; Peters and Van Slyke state that prolonged protein under-nutrition produces a deficit in blood plasma protein; during the acute stage of cholera the dehydration causes a relative concentration of plasma proteins in spite of both the initial deficit and of the increased protein katabolism caused by anhydraemia; as soon as the acute stage is over, plasma volume is restored much more rapidly than the protein content, with a resultant fall in plasma protein in many cases below the oedema level; since plasma proteins in females are on the average higher than in males, it is reasonable to expect that this oedema level would be reached more frequently in males than in females; this is the importance of the high incidence of oedema amongst males pointed out at the beginning of this paper. Additional evidence on this point is the fact that although the oedema disappeared spontaneously, its disappearance was hastened by an increased meat diet; and in connection with this it is interesting to note that the patients—all Chinese who are not generally big meat eaters—were without exception eager to receive the increased meat ration.

One other aspect of this nutritional problem is that on the whole, the women patients were drawn from a better nourished class of the community; the males were for the most part from the poorest of Hong Kong's poor, the street sleepers, and for this reason also one would expect to find a sex difference in average plasma protein values, which, on the malnutrition theory, would reflect itself in a high male oedema incidence. We feel that both these nutritional factors played basic parts in the production of the oedema.

There is one other factor which we shall now discuss but concerning which we have only indirect evidence; we shall show later how in a number of cases the vitamin B_1 content of the body was greatly reduced by the loss of body fluids. It is reasonable to suppose that this is liable to happen to any of the body's stores of soluble active principles especially if the abnormal conditions call for a great production and use of such substances. The outstanding feature of cholera is the intense dehydration, and this condition lasting for days must throw a tremendous strain on the mechanism which maintains the water balance in the body. Foremost amongst the organs involved are the kidneys; they have to perform their secretory activities under the following most unfavourable conditions, a low blood pressure, a sluggish viscid blood flow through the glomeruli and a marked decrease in fluid available to act as solvent for excretory products. We shall see later how many of the kidneys fail to contend with these unfavourable conditions; those that do survive the ordeal of the acute stage show the effects during convalescence; during this period they perform their functions with every sign of very fatigued organs; the passive process of glomerular filtration is performed normally but the active tubule functions are greatly reduced, resulting in the low concentration urines which we have seen characterise all types of convalescent cholera urines.

Swingle (1934, a, b; 1936, a) and his co-workers have produced abundant evidence as to the part played by the suprarenal cortex in the maintenance of water balance in the body. Unfortunately we were unable to obtain any supplies of supra-renal extract in Hong Kong so this theory could not be put to the test, but we suggest that if there is any reason to believe that cholera results in a depletion of stores of B_1, there is every reason to expect a marked depletion of such water-balance hormone as that of the supra-renal cortex by usage as well as actual loss, so that when the nutritional factors lead to the appearance of oedema, the absence of cortex hormones makes it impossible for the body to deal efficiently with these abnormal collections of fluid. We thus consider that these patients are in this respect comparable to the decorticated dogs of Swingle's experiments and that this type of oedema so frequent in the Hong Kong cholera epidemic of 1937, was primarily due to a loss of plasma proteins from the blood already poor in these, owing to malnutrition, and its persistence was due to the fatigue of the normal water balance mechanism of the body consequent on the strain of the acute stage.

Second Type of Oedema.

We must now turn to the second type of oedema. Owing to the nature of this paper the clinical aspect of these cases will be but summarily dealt with. The oedema we have already described; concerning the tendon reflexes, knee and ankle jerks were invariably absent, biceps, triceps and supinators often absent; muscles were flabby

except when oedematous; and atonic muscle wasting was obvious especially when the upper limbs were involved; calf muscles were very frequently tender and motor disturbances ranging from diminution of strength of grip to complete paralysis of arms and legs with attendant wrist and foot drop; in some of those able to walk a typical cock-step gait was present, and it was interesting to compare these cases with those having the other type of oedema, for in these latter the interference with efficient muscular action was not neuromuscular but purely mechanical, due to the marked local accumulation of tissue fluid. In most of these cases, various blowing, murmur and tapping sounds could be heard when the stethoscope was applied over the brachial, femoral or dorsalis pedis arteries, less frequently over the popliteal, but in no case could we detect the sounds over the facial or temporal arteries. Aarlsmeer's test was positive in a number that we investigated.

The following notes on two of these cases illustrate these points:

No. 579. Male, aged 28, a coolie (opium smoker and street sleeper), admitted on the 25th August, 1937, transferred to the convalescent ward on the 4th September; oedema appeared about this time. On the 18th his feet, legs, hands and chest wall were markedly oedematous; where the absence of oedema made examination of the limb muscles possible they were found to be flabby, atonic and atrophied. No cardiac enlargement was detected, heart sounds were normal and there was no visible pulsation in the neck. B.P. 104/48. Lungs clear. Knee and ankle jerks could not be elicited on either side, both plantar reflexes were flexor, abdominal and biceps reflexes were easily elicited but triceps jerks were absent on both sides. No cutaneous sensory loss could be demonstrated.

On the 25th September there was marked oedema of feet ankles and legs, more marked on the right than on the left; there was no oedema of the hands. Muscles of the arms and legs were tender to pressure, but no pain was elicited on strongly pinching the Achilles tendons. Wasting of the arm muscles was noticed. Knee and ankle jerks still negative and both biceps jerks had become negative.

On the 27th September, pulsation was visible in the neck and arterial sounds were present over the brachial, femoral and dorsalis pedis arteries, but not over the popliteal, facial or temporal. B.P. 102/56. Pulse 96. Patient unable to walk. 5 mgms. of Betaxin administered intravenously. Pyruvic acid 1.91 mgms. %.

On the 28th of September, oedema present on feet only more marked on left than on right. Patient usable to lift either feet or legs off the bed. Muscles atonic and flabby. Knee and ankle jerks still absent, biceps jerks again positive on both sides. Arterial sounds present over femoral and dorsalis pedis arteries. B.P. 128/78. Pulse 82. Blood pyruvic acid 0.90 mgms. %.

On the 1st of October oedema had entirely disappeared leaving the typical loose post-oedematous skin. Patient able to lift both legs off the bed. No change in tendon reflexes; muscles still flabby and atonic. Faint sounds heard over the brachial arteries only.

On the 4th of October muscle strength was improving and the patient was able to walk; he was discharged to another hospital for continuation of treatment.

No. 623. Male, aged 32, occupation unknown. Admitted on the 26th of August, convalescent on the 30th and oedema appeared a few days later. Almost immediately afterwards he began to walk with a cock-step gait. On the 18th of September oedema had spread from feet to legs and thighs, hands and arms. Muscles where they were not marked by the oedema were atonic and flabby. B.P. 128/64. Pulse 100. Visible precordial pulsation.

25th of September, oedema increased. Unable to lift legs off bed. Muscles very tender on pressure and on movement. Tendon reflexes absent. Cutaneous sensation normal. Precordial pulsation still visible.

27th of September. Unable to sit up. Muscles all over body painful on pressure. Pulsation visible in neck. Arterial sounds present over brachial and femoral arteries only. B.P. 132/78. Pulse 108. Blood pyruvic acid 2.19 mgms. 5 mgs. of Betaxin administered intravenously.

28th of September. Oedema decreased. Skin over legs wrinkled. Muscles atonic and flabby but less painful; cannot lift feet off bed. Tendon reflexes still absent. Arterial sounds fainter and only elicited by deep pressure of stethoscope bell. B.P. 128/92. Pulse 64. Blood pyruvic acid 1.07 mgms. %.

1st October. Oedema entirely disappeared. Muscle weakness increased. Wasting of hypothenar; foot and wrist drop present with clawing of hands. Tendon reflexes still absent. Arterial sounds still present.

2nd October. Blood pyruvic acid 1.02 mgms. %. 5 mgms. Betaxin given intravenously.

3rd October. Blood pyruvic acid 0.26 mgms. %.

4th October. Muscle power improving slowly but foot and wrist drop still present and these remained till patient was discharged to another hospital for further treatment.

In all these cases a diagnosis of sub-acute beri-beri was made. They differed from the other cases in the type of oedema, in the fact that it did not disappear spontaneously, in the progressive circulatory disturbances and progressive muscular weakness, in the marked muscle tenderness and in the progressive peripheral nerve involvement especially of the long nerves; in the high blood pyruvic acid content and in the remarkable way the oedema and muscle weakness responded to B_1 therapy.

SUMMARY.

1. A brief statement is made concerning the 1937 cholera epidemic in Hong Kong, and concerning the methods of investigation used.

2. Two types of oedema occurring among the convalescents are described and the onset, termination and sex incidence discussed.

3. Methods of investigating the cause of oedema including blood pressures and urine and blood analyses are given in detail.

4. Causes of oedema are discussed and conclusions reached that the oedema in the larger group of cases was due to malnutrition in general, and the oedema in the smaller group was due to a particular nutritional deficiency, namely avitaminosis B_1.

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REFERENCES.

CULLEN, G. E. - - - - (1929) An improved form of the quinhydrone electrode. *Jl. Biol. Chem.*, *83*, (3), 535.

FOLIN, O. - - - - - - (1917) Recent biochemical investigations on blood and urine. Their bearing on clinical and experimental medicine. *J. Am. Med. Assoc.*, *69*, *1209*.

GREENBURG, D. M. - - (1929) The colorimetric determination of the serum proteins. *Jl. Biol. Chem.*, *82*, *545*.

LING, K. D., AND CHENG, S. Y. - - - - (1937) Determination of Blood Urea. A modified micro-method using 0.2cc of blood. *Caduceus*, *16*, (4), *154*.

LING, K. D., AND CHENG, S. Y. - - - (1937A) Deetermination of non-protein nitrogen. A modification of Folin's micro-method using 0.2 cc of blood or less. *Caduceus*, *16*, (4), *149*.

MILLAR, W. G. - - - (1925) Observations on the haematocrite method of measuring the volume of erythrocytes. *Q. Jl. Physiol.*, *15*, *187*.

PATTERSON, JOCELYN - (1928) The determination of chlorides in body fluids. *Bioch. J.*, *22*, *758*.

PETERS, J. P., AND VAN SLYKE, D. D. - - (1931) Quantitative Clinical Chemistry. Interpretaions. *Bailliere Tindall & Co.*

PETERS, R. A., AND THOMPSON, R. H. S. - (1934) Pyruvic Acid as an intermediary metabolite in the brain tissues of avitaminotic and normal pigeons. *Bioch. Jl.*, *28*, (1), *916*.

RIDE, LINDSAY - - - (1936) On the sedimentation of Red Blood Cells. *Caduceus*, *15*, (3), *115*.

RIDE, LINDSAY, LING, K. D., LIM, E. Q., AND QUEK, C. K. (1936) Physiological blood consants in Hong Kong Chinese. *Caduceus*, *15*, (4), *189*.

ROGERS, LEONARD - - (1921) Bowel diseases in the tropics. *Oxford Medical Publications*.

SCHMIDT, C. - - - - (1850) Charakteristik der Epidemischen Cholera. Leipsig.

SELLARDS, A. W., AND SHAKLEE, A. O. - - - (1911) Indications of acid intoxication in Asiatic Cholera. *Philippine Jl. of Sc.*, *6*, (1), *53*.

SWINGLE, W. W., PFIFFNER, J. J., VARS, H. M., AND PARKINS, W. M. - - (1934) The effect of fluid deprivation and fluid intake upon the revival of dogs suffering from adrenal insufficiency. *Am. Jl. Physiol., 108, 144.*

SWINGLE, W. W. PFIFFNER, J. J., VARS, H. M. AND PARKINS, W. M. - - (1934A) The effect of NaCl administration upon adrenalectomized dogs not receiving extract. *Ibid., 108, 159.*

SWINGLE, W. W., PFIFFNER, J. J. VARS, H. M., AND PARKINS, W. M. - - (1934B) The relation between blood pressure, blood urea and fluid balance of the adrenalectomized dog. *Ibid., 108, 428.*

SWINGLE, W. W., PARKINS, W. M. AND TAYLOR, A. R. - (1936) Experiments on intact and adrenalectomized dogs subjected to sodium and chloride depletions by intraperitoneal injections of glucose. *Ibid., 116,* (2), *430.*

SWINGLE, W. W., PARKINS, W. M. AND TAYLOR, A. R. - (1936A) Relation of serum sodium and chloride levels to alterations of body water in the intact and adrenalectomized dog and the influence of adrenal cortical hormone upon fluid destruction. *Ibid., 116,* (2), *938.*

THE USE OF 1.5. DIPHENYLTHIOCARBAZONE (DITHIZONE) IN THE DETERMINATION OF TRACES OF ZINC IN BIOLOGICAL MATERIALS.

by

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Diphenylthiocarbazone in chloroform or carbontetrachloride has been used for several years as a medium for extracting minute quantities of lead from the ash of biological materials. Recently Sylvester and Hughes have adapted the use of this reagent for the extraction of zinc. Since the publication of Sylvester and Hughes' suggestions the author has made numerous checks on the accuracy of the method and from the experience gained in over four hundred analyses of biological ash it has been possible to introduce several small modifications and refinements in the technique. These are described in the following pages. The method has been found capable of giving accurate results and is considerably quicker than any other method involving separation of the zinc by preciptation.

Method.

A suitable weighed quantity of the oven-dry material for investigation contained in a platinum basin is burnt off as far as possible over a bunsen flame and finally ashed in an electric muffle furnace at a temperature just below 450° C. Oxidation of the organic matter is assisted if necessary by addition of a few drops of nitric acid. Care should be taken to avoid excess of nitric acid as in the presence of chlorides in the ash, platinum tends to be dissolved with the risk of interference in the subsequent analysis. During ashing care should be taken to prevent rapid oxidation as this is likely to produce local temperatures considerably in excess of 450° C. with resulting loss of zinc. After weighing, the carbon-free ash is dissolved in 5 ml. of 5N (constant boiling point) hydrochloric acid, and, after solution has been effected by heating, transferred to a separating funnel containing 10 ml. of 5N ammonium acetate (386 g./litre). The last traces of soluble ash together with any insoluble residue of silica are heated to boiling with 10 ml. of distilled water and the whole transferred to the separating funnel with the aid of a further 20 ml. of distilled water used fractionally. Rogers and Gall (1937) have shown by spectrographic means that when the ash from vegetable matter is treated with hydrochloric acid a portion of the zinc is sometimes not extracted. For this reason the whole contents of the basin should be transferred to the separating funnel, and the basin, after thorough washing, tested with a small quantity of the dithizone solution.

The solution in the separating funnel is shaken with successive minimal quantities of a 0.1 per cent. copper and zinc-free solution of diphenylthiocarbazone in chloroform, prepared according to the method described by Sylvester and Lampitt (1935). The complete extraction of the zinc is indicated by the colour of the chloroform layer remaining green after shaking. The chloroform extracts combined in a second separating funnel are shaken with 15 ml. of a mixture of 350 ml. of 5N ammonium acetate and 200 ml. of 5N hydrochloric acid made up to one litre, and then in a third funnel washed with several portions of 10-20 ml. of distilled water to remove ammonium salts. In transferring the chloroform layer from one separating funnel to another the remaining traces of colour should be washed out with successive minimal portions of chloroform. The washed extracts are finally collected in a fourth funnel and thoroughly shaken with two successive small quantities of 0.5N (diluted from 5N) hydrochloric acid to extract the zinc. After separation and thorough washing with chloroform to remove the last traces of green colour, the acid solution of the zinc, collected in a 50 ml. beaker together with washings is evaporated to dryness over a very low flame and finally on the water bath if necessary, although this precaution may be omitted if great care is taken not to overheat the beaker. Small residual traces of organic matter are removed by treatment with a few drops of pure hydrogen peroxide, followed by careful evaporation to dryness making sure that all hydrogen peroxide is driven off. If sufficient care is always taken to wash thoroughly both with chloroform and water at the appropriate stages, the additional use of perchloric acid, as suggested by Sylvester and Hughes, is rarely necessary. This is a great advantage since prolonged heating is required to eliminate the last traces of perchloric acid. It has been found necessary to distil a small quantity of hydrogen peroxide prior to use as certain brands tested have been found to leave a slight residue which chars.

To the cooled residue 2 ml. of 20 per cent. acetic acid are added, followed by a small crystal of pure potassium iodide and a few drops of recently prepared 2-5 per cent. starch solution. Any blue colour which may develop after standing for half a minute should be very carefully titrated away with 0.002N sodium thiosulphate. A slight excess (usually two or three drops) of 1 per cent. potassium ferricyanide is next added whereupon iodine is quantitatively liberated according to the equation :—

$$2K_3Fe(CN)_6 + 2KI + 3ZnSO_4 = K_2Zn_3(Fe(CN)_6)_2 + 3K_2SO_4 + I_2.$$

Much of the iodine is occluded in the precipitate of zinc ferricyanide giving it a dark colour. The 0.002N sodium thiosulphate is then added drop by drop from a micro-burette, the end-point being indicated by the colour of the precipitate changing to white. The final stages should be followed with a hand lens such as a linen

tester. The solution in the beaker should be rolled round the bottom of the beaker during the titration and not violently agitated. After the titrated solution has stood for a few minutes there is a slight return of the blue colour: this is due to secondary reactions and is ignored. In the titration, 0.51 ml. of 0.002N sodium thiosulphate is equivalent to 0.1 mg. of zinc.

Purity of Reagents.

It is necessary that the reagents used should be free from zinc. Glacial acetic acid and hydrochloric acid may be readily freed from zinc and other metallic impurities by distillation. The ammonium acetate which has been found to be one of the major sources of impurity may be freed from zinc and certain other undesirable metals by shaking the prepared solution with successive portions of dithizone solution. It is not necessary to boil off the residual traces of chloroform although the colour should be well washed out. One batch of ammonium acetate tested was found to contain 83 parts per million of zinc and heavy traces of copper, tin and lead; another batch contained only 1 part per million of zinc. If these precautions are observed the blank should not be detectable in terms of .002N thiosulphate.

Sources of Error.

One of the principal sources of error in the determination is that which arises from the gradual change in the strength of the 0.002N sodium thiosulphate. Some authors recommend that a freshly prepared solution should be used if the interval of time between titrations is more than a few hours, but this can be conveniently avoided by titrating the 0.002N sodium thiosulphate in triplicate with standard potassium iodate every third or fourth day. From a prepared smoothed graph showing the change of strength with time, the correct strength for any day corresponding to a determination can be obtained with accuracy. The 0.002N sodium thiosulphate was found to change approximately 0.5 per cent. per day during the first fourteen days after which it was discarded and a fresh quantity prepared from a stock solution of 0.2N strength. Finally it should be mentioned that ashing should be carried out in a platinum basin. Silica dishes after they have become etched are definitely unsuitable where small quantities of zinc are involved.

Preparation of the Diphenylthiocarbazone.

Owing to unforeseen difficulties it was found necessary to prepare a supply of the diphenylthiocarbazone. This was found to be an extremely simple matter and a quantity of this expensive chemical sufficient for many weeks' work was prepared in a few hours. In view of the fact that Fischer and Besthorn (1881) recorded the preparation of this subtance nearly sixty years ago a copy of the

original paper is sometimes difficult to acquire, consequently a useful purpose is served by giving a short description of the method.

A small quantity of the ether-washed and pressed precipitate of the phenylhydrazine salt of phenylhydrazinedithiocarbonic acid, which is formed by slowly adding phenylhydrazine to a cooled solution of carbondisulphide in ether, is carefully heated for ten minutes at 100-110° C. in the bottom of a small beaker. The powder melts, evolves sulphuretted hydrogen and produces, with slight darkening, a thick oily mass consisting of 1.5 diphenylthiocarbohydrazide, aniline and l. phenylthiosemicarbazide. A small quantity of alcoholic potash is then added and the whole boiled gently for five minutes, adding a little more alcohol if necessary. The 1.5. diphenylthiocarbazone, together with some l. phenylthiosemicarbazide, is precipitated from this solution by the addition of dilute sulphuric acid. The desired compound is obtained by taking up the washed and pressed precipitate with 5 per cent. ammonia and reprecipitating with dilute sulphuric acid.

Accuracy of the Method.

Sylvester and Hughes (1936) recommend the use of Lang's method of estimation for amounts of zinc not exceeding 0.3 mg., which suggests that above this limit the values obtained are not accurate. It was thought desirable to ascertain if larger quantities of zinc could be titrated safely by this method. The results are tabulated below:

TABLE I.

Zinc taken mg.	Zinc found mg.
.0227	.0230
.0455	.0469
.1137	.1140
.1344	.1364
.2274	.2275
.364	.363
.909	.927
1.137	1.156
2.274	2.331
4.542	4.586

These figures agree remarkably well for amounts of zinc ranging from .02—4.5 mg. This corresponds to 23—4500 parts per million on a 1 g. sample. Since the amount of .002N sodium thiosulphate equivalent to .02 mg. of zinc is 0.117 ml., it will be seen that even a fifth or a tenth of this amount can be titrated with fair accuracy.

A further idea of the accuracy of the titration may be gathered from the figures given in the following tables where the values, 0.2277, 0.2296, 0.2279 and 0.2279 mg. were obtained in four occasional titrations of 1 ml. of zinc solution containing theoretically 0.2274 mg. of zinc.

In order to test the accuracy of the method of extraction, 1 ml. of a zinc sulphate solution containing 0.1802 mg. of zinc was put through the whole process of extraction. The amount of zinc recovered was 0.1796 mg. Table 5 also shows that .2268 mg. were recovered when .2279 mg. were put through the whole process of extraction.

Effect of Addition of Bismuth.

Bismuth is extracted along with zinc but the amount of bismuth normally occurring in biological material is usually so extremely small that no provision for its elimination is called for. Nevertheless it was thought desirable to ascertain the effect of various amounts of bismuth on the titration of a small amount of zinc. The figures obtained are given below:

TABLE 2.

Bismuth added mg.	Zinc found mg.
—	.2277
.01	.2290
.10	.2296
.40	.2310
1.00	.2392
2.00	.2320
3.50	.2353
4.00	.2390

Amounts of bismuth greater than 2.0 mg. produced the characteristic yellow colour on addition of the potassium iodide thus causing some uncertainty in determining the end-point.

It would appear from the above figures that a serious error is not likely to occur from the inclusion of bismuth even to the extent of a few milligrams.

Effect of Addition of Lead.

The lead compound of dithizone is not extracted from the original solution under the defined conditions of acidity so that traces of lead in the material do not affect the accuracy of the results. However in view of the fact that traces of lead may be introduced as an impurity in the reagents used, the effect of this element on the final titration has been tested.

The presence of even 1 mg. of lead in the final material does not affect the accuracy of the determination to an important extent.

TABLE 3.

Lead added mg.	Zinc found mg.
—	.2296
.01	.2312
.10	.2290
.40	.2312
1.0	.2331

Effect of Addition of Cadmium.

Cadmium is extracted along with the zinc and any present in the original material will find its way into the final material for titration. Cadmium occurs in biological material only in very small traces and always to a much smaller extent than zinc. For this reason it is not considered necessary to make what is at best a very difficult separation. The effect of cadmium on the accuracy of the titration is shown in the following table:

TABLE 4.

Cadmium added mg.	Zinc found mg.
—	.2279
.01	.2274
.05	.2314
.10	.2400
.20	.2451
.30	.2451
.40	.2471
.50	.2500
1.00	.2690

The presence in the zinc solution of even an equivalent amount of cadmium has the effect of increasing the titre by only about 10 per cent. In view of the fact that no guarantee was forthcoming regarding the absence of zinc in the cadmium salt used the above figures represent a maximum effect. The presence of cadmium can be shown by the use of 3. nitro-4-hydroxyphenylarsonic acid, which is stated by Pavelka and Kolmer (1930) to be specific for cadmium.

Effect of Copper.

Although copper if present is extracted from the original solution along with the zinc it is effectively separated when the chloroform

solution is finally extracted with 0.5N hydrochloric acid. The following table shows that 1.0 mg. of copper can be separated reasonably well from 0.2 mg. of zinc by this means:

TABLE 5.

Zinc present mg.	Copper added mg.	Zinc found	
		before extn. mg.	after extn. mg.
.2274	—	.2279	.2268
.2274	0.10	.2350	.2291
.2274	1.00	poor end-point	.2189

Effect of Other Metals.

The alkali metals, alkaline earths, aluminium, iron and manganese do not form compounds with dithizone and consequently need not be considered. Silver forms a compound with dithizone but when present it is eliminated as the insoluble chloride, when the final dithizone extract is treated with 0.5N hydrochloric acid. Tin, thallium and mercury form compounds but these metals are hardly ever present in biological material and may be safely ignored. Nickel and cobalt do not appear to interfere. Little appears to be known about titanium which is occasionally found in biological material, but in a few experiments which have been made the indications are that it is not extracted. Precise information on this point was rendered impossible through lack of a source of pure titanium. Arsenic, antimony, molybdenum and vanadium have been detected occasionally in very small amount in biological material, but it is not considered likely that such small traces of these metals would interfere with the titration to an important extent.

REFERENCES.

BESTHORN, E., AND FISCHER, E. - - - - - (1881) *Annalen, 212, 316.*

FISCHER, H. - - - - - (1930) *Mikrochem, 8, 319.*

HIBBARD, P. L. - - - (1934) *Ind. & Eng. Chem., Anal. Ed., 6, 423.*

LANG, R. - - - - - - - (1929) *Z. Anal. Chem., 79, 161;*

LANG, R. - - - - - - - (1933) *ibid., 93, 21.*

PAVELKO, F., AND KOLMER, E. - - - - (1930) *Mikrochem, 8, 277.*

ROGERS, L. H., AND GALL, O. E. - - - - (1937) *Ind. & Eng. Chem., Anal. Ed., 9, 42.*

SYLVESTER, N. D., AND HUGHES, E. B. - - - (1936) *Analyst, 61, 734.*

SYLVESTER, N. D., AND LAMPITT, L. H. - - - (1935) *ibid., 60, 376.*

THOMPSON, P. K. - - (1925) *J. Ind. Hyg., 7, 358.*

OBSERVATIONS ON TWO OF THE SULPHANILAMIDE GROUP IN ACUTE MENINGOCOCCAL MENINGITIS.

by

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The following clinical investigations were undertaken in an endeavour to assess the therapeutic value of two members of the sulphanilamide group of chemo-therapeutic agents in the treatment of acute meningococcal meningitis. The dosage, being mainly determined by strict economy, was purposely kept on a low level.

In recent literature great therapeutic value is claimed for sulphanilamide in meningitis of this type but it would appear almost impossible to assess the true efficiency of any one particular agent where mixed treatment is being adopted. In this contribution it is not proposed to refer to all recent articles bearing on the matter in question. Schwentker *et al.* (1937) treated 10 cases with intrathecal and subcutaneous injections of sulphanilamide alone—only 1 case died. Eldahl (1938) treated 12 cases with sulphanilamide alone—intrathecally and intramuscularly—3 cases died.

Banks (1938) gives a very excellent comparative survey of the treatment of 113 cases of acute meningococcal meningitis. Of the 113 cases 16 selected were treated with streptocide by oral administration alone and 15 recovered—a surprisingly good result. He recommends high initial dosage and suggests that sulphanilamide is effective only in the acute and early stage. In 59 unselected cases combined treatment with serum and sulphanilamide the fatality rate was 11.8 per cent.

In the present series 23 Chinese cases were treated with sulphanilamide solution only and none was administered by mouth.

Diagnosis.

In all cases this was confirmed by finding the meningococcus in direct smear and culture. No attempt was made to group the organism. Preparations used:—

Soluseptasine (May & Baker) 5%.

Streptocide (Evans, Sons Lescher & Webb) 2.5%.

Method of administration:—

Either by intravenous, intramuscular or intrathecal route, or in combination.

Lumbar or cisternal puncture was done daily as necessary in all cases.

Four tables arranged in decades are shown and only essential clinical data are given-particular note being taken of the time when the meningococci disappeared from the C.S.F. Complete daily examinations of the fluid were made but details are omitted.

TABLE I (*1st decade—8 cases, 2 recoveries*).

Case	*Sex & Age*	*Illness before admission*	*C.S.F.* — *On admission*	*Soluseptasine 5%*	*Streptocide 2.5%*	*Total dose*	*Result*
1.	M. 5 yrs.	Uncertain	Turbid. Meningococci + + Polymorphs 515 per cmm.	10 c.c.		10 c.c.	Died 24 hours after admission
2.	M. 5 yrs.	Uncertain	Turbid. Meningococci + + Polymorphs 4,480 per cmm. 10 days later—Sterile. Polymorphs 1,200 per cmm.		3 c.c. in 10 c.c. saline I.T. daily 9 days 5 c.c. I.M.I. daily 9 days	72 c.c.	Died—20th day
3.	M. 9 yrs.	4 days	Turbid. Meningococci + + Polymorphs 9,520 per cmm. 3 days later—Sterile. Polymorphs 1,180 per cum.		5 c.c. in 10 c.c. saline I.T. 5 c.c. I.M.I. for 10 days	55 c.c.	Recovery complete
4.	M. 2 yrs.	6 days	Turbid. Meningococci + + Polymorphs 2,980 per cmm. 9 days later—Sterile. Polymorphs 750 per cmm.		5 c.c. I.M.I.	50 c.c.	Died—28th day
5.	M. 5 yrs.	12 days	Purulent. Meningococci + + Polymorphs 36,800 per cmm.		10 c.c. I.M.I.	30 c.c.	Died—4th day
6.	F. 6 yrs.	10 days	Turbid. Meningococci + + Polymorphs 280 per cmm.		10 c.c. I.M.I.	60 c.c.	Died—9th day
7.	M. 7 yrs.	10 days	Turbid. Meningococci + + Polymorphs 2,170 per cmm.		5 c.c. in saline I.T. 5 c.c. I.M.I.	20 c.c.	Died in 24 hours
8.	F. 4 yrs.	2 days	Turbid, slight. Meningococci + + Polymorphs 140 per cmm. 3 days later—Sterile. Polymorphs 100 per cmm.		5 c.c. I.M.I.	50 c.c.	Recovery complete

TABLE II (*2nd decade—2 cases, 2 recoveries*).

Case	*Sex & Age*	*Illness before admission*	*C.S.F.* *On admission*	*Soluseptasine 5%*	*Streptocide 2.5%*	*Total dose*	*Result*
1.	M. 15 yrs.	3 days	Turbid Polymorphs 3,650 per cmm. Meningococci + + 4 days later— Polymorphs 910 per cmm. Sterile		5 c.c. with 10 c.c. saline I.V.I. + 5 c.c. I.M.I. daily for 10 days	100 c.c.	Complete recovery
2.	F. 11 yrs.	5 days	Turbid Polymorphs 2,420 per cmm. Meningococci + + 4 days later— Polymorphs 180 per cmm. Sterile		10 c.c. I.M.I. daily	110 c.c.	Complete recovery

TABLE III (*3rd decade—7 cases, 3 recoveries*).

Case	*Sex & Age*	*Illness before admission*	*C.S.F.* — *On admission*	*Soluseptasine 5%*	*Streptocide 2.5%*	*Total dose*	*Result*
1.	M. 30 yrs.	Uncertain	Turbid. Meningococci ++ Polymorphs 44,200 per cmm. 5 days later—Sterile Polymorphs 19,800 per cmm.	10 c.c. I.V.I. for 5 days		50 c.c.	Recovery
2.	F. 21 yrs.	3 days	Polymorphs 60 per cmm. Meningococci ++ 3 days later—Sterile Polymorphs 40 per cmm.	10 c.c. I.V.I. for 12 days		120 c.c.	Recovery
3.	F. 22 yrs.	Uncertain	Turbid. Meningococci ++ Polymorphs 10,800 per cmm.	5 c.c. in 20 c.c. saline		10 c.c.	Died within 24 hours
4.	F. 28 yrs.	3 days	Purulent Polymorphs 30,600 per cmm. Meningococci ++ 3 days later—Sterile Turbid Polymorphs 4,450 per cmm.	10 c.c. I.V.I. for 4 days then 10 c.c. in 10 c.c. saline I.T. for 8 days followed by—	10 c.c. I.M.I. for 2 days 5 c.c. I.V.I. daily for 9 days	105 c.c.	Recovery
5.	M. 26 yrs.	Uncertain	Turbid. Meningococci ++ Polymorphs 2,730 per cmm. No change in C.S.F. up to time of death		5 c.c. with 20 c.c. I.T. plus 5 c.c. I.M.I. daily	50 c.c.	Died—4th day
6.	M. 26 yrs.	Uncertain	Turbid. Meningococci ++ Polymorphs 36,000 per cmm. 6 days later—Sterile Turbid Polymorphs 3,000 per cmm.		10 c.c. I.M.I. plus 5 c.c. with 20 c.c. saline I.T. daily for 14 days	210 c.c.	Died—7th week
7	F. 24 yrs.	Uncertain	Turbid. Meningococci ++ Polymorphs 2,530 per cmm. 4 days later— Turbid. Meningococci ++ Polymorphs 1,510 per cmm.		5 c.c. with 15 c.c. saline I.T. plus 5 c.c. I.M.I. daily	60 c.c.	Died—4th day

TABLE IV (*4th decade—6 cases, 1 recovery*).

Case	*Sex & Age*	*Illness before admission*	*C.S.F.* — *On admission*	*Soluseptasine 5%*	*Streptocide 2.5%*	*Total dose*	*Result*
1.	M. 33 yrs.	Uncertain	Turbid. Meningococci + + Polymorphs 366 per cmm.	10 c.c. with saline 20 c.c.		15 c.c.	Died within 24 hours
2.	F. 31 yrs.	5 days	Turbid Polymorphs 70 per cmm. P.M. Diagnosis		5 c.c. with 20 c.c. saline I.T. 10 c.c. I.M.I.	15 c.c.	Died within 48 hours
3.	F. 43 yrs.	Uncertain	Turbid. Meningococci + + Polymorphs 8,280 per cmm. 6 days later— Polymorphs 2,570 per cmm. Meningococci ††		5 c.c. with 10 c.c. saline I.T. plus 10 c.c. I.M.I. daily	95 c.c.	Died—6th day
4.	M. 38 yrs.	Uncertain	Turbid. Meningococci + + Polymorphs 6,840 per cmm.		10 c.c. I.M.I.b.d. (1st day) 10 c.c. with 20 c.c. saline I.T. plus 5 c.c. I.M.I. (2nd day)	30 c.c.	Died within 48 hours
5.	M. 40 yrs.	20 days	Turbid. Meningococci + + Polymorphs 617 per cmm. 3 days later—Sterile. Polymorphs 312 per cmm.		5 c.c. with 15 c.c. saline I.T. Then 10 c.c. I.M.I. daily for 10 days	120 c.c.	Died—10th day
6.	F. 32 yrs.	10 days	Turbid. Meningococci + + Polymorphs 1,530 per cmm. 3 days later—Sterile. Polymorphs 886 per cmm.		5 c.c. with 15 c.c. saline I.T. for 10 days	50 c.c.	Recovery

Remarks.

This investigation was undertaken with the full consciousness that economy in the use of the drug was essential. Both large dosage and medication by mouth were refrained from.

The main reason for the very unsatisfactory results would obviously appear to be the low dosage of the drug used. The highest total in any one case was approximately 200 c.c.—compare this with the huge initial dose given by Willien (1938) namely 500 c.c. for a normal sized adult.

Allot's (1938) investigations on the sulphanilamide content of the C.S.F. during treatment of meningococcal meningitis suggest that adequately high dosage is necessary. He states however that there is a very considerable variation in the rate with which an effective value is reached in the C.S.F.

The fatality rate of 65.2% is excessively high. One reason is undoubtedly the difficulty of early diagnosis and hospitalisation in this part of the world. It is extremely difficult to obtain reliable infromation as to the duration of sickness before admission to hospital with the result that figures on such a point are not to be fully trusted.

The following additional Tables V and VI for comparison have been compiled from 65 Chinese cases of acute meningococcal meningitis under the care of Dr. C. W. Lam and treated by anti-serum alone.

Lumbar puncture was performed as usual and anti-serum was given by intrathecal route only. Detailed descriptions of cases have been omitted.

TABLE V.

Decade	Males	Recovered	Died	Average total of anti-serum per patient
1st	10	6	4	70 c.c.
2nd	6	5	1	
3rd	4	3	1	
4th	1	—	1	
Total	21	14	7	

Fatality Rate=33.3%.

TABLE VI.

Decade	Females	Recovered	Died	Average total of anti-serum per patient
1st	14	7	7	
2nd	13	10	3	
3rd	12	7	5	
4th	1	1	—	80 c.c.
5th	3	2	1	
6th	1	1	—	
Total	44	28	16	

Fatality Rate = 36.3%.

Remarks.

The fatality rates are not excessively high considering certain grave disadvantages under which treatment has to be carried out in the case of Chinese patients.

The average amount of anti-serum per patient was small. After ten years' experience of treating meningococcal meningitis in Hong Kong there is every evidence that the locally produced polyvalent anti-serum which contains 15 different strains, 14 of local origin, is a potent therapeutic agent. The anti-serum is prepared by Dr. A. V. Greaves, in charge of the Government Bacteriological Institute, Hong Kong. The efficacy of this anti-serum is maintained by the continuous watchfulness for new local strains of the meningococcus which are added to replace older strains.

Summary.

1. 23 Chinese cases of acute meningococcal meningitis of confirmed diagnosis have been treated with soluseptasine 10% and streptocide 2.5%.

2. No toxic symptoms due to the sulphanilamide were noted. Streptocide was given intravenously without untoward effect.

3 The cases are divided into 4 decades as shown in Tables I, II, III and IV.

4. The fatality rate of 65.2 per cent. is excessively high. The main reason is presumed to be the low dosage of sulphanilamide.

5. Two additional Tables V and VI are shown for comparison. They give condensed results and fatality rates in 65 Chinese cases of acute meningococcal meningitis treated by anti-serum alone.

We wish to express our gratitude to the Director of Medical Services and to M.O. in-charge for all facilities afforded us at the Queen Mary Hospital.

Our thanks are due to the ever willing help of Dr. K. D. Ling, Dr. H. T. Wu, Dr. W. Heng and Dr. W. W. Yeung also to Dr. C. W. Lam for his anti-serum cases.

Finally we gratefully acknowledge the kindness of Messrs. May and Baker and Evans Sons Lescher and Webb for a certain amount of the drugs supplied free of cost.

REFERENCES.

SCHWENTKER, F. F., GELMAN, S., AND LONG, P. H. - - - (1937) The Treatment of Meningococcal Meningitis with Sulfanilamide. *J. Amer. Med. Ass., 108, 1047.*

ELDAHL, A. - - - - - - - (1938) Intrathecal Sulphanilamide in Meningococcal Men ingitis. *Lancet, I, 712.*

BANKS, H. S. - - - - - - (1938) Serum and Sulphanilamide in Acute Meningococcal Meningitis. *Lancet, II, 7.*

WILLIEN, L. J. - - - - - - (1938) Sulfanilamide Therapy in Meningococcal Meningitis. *J. Amer. Med. Ass., 110, 630.*

ALLOT, E. N. - - - - - - (1938) Sulphanilamide Content of Cerebro-Spinal Fluid During The Treatmen of Meningococcal Meningitis. *Lancet, II, 19.*

A PHOTOGRAPHIC RECORD OF SCURVY IN SHANGHAI

by

E. Vio and S. T. Hsiu.

During the period of hostilities and under quite unavoidable circumstances scurvy broke out in a Municipal Gaol and remained prevalent until the end of January 1938. Between October 16th, 1937 and January 28th, 1938, 431 cases were admitted into the Hospital whilst about 700 mild cases treated in the O.P.D. Of these 17 deaths occurred and the condition was probably indirectly responsible for a number of deaths due to other causes.

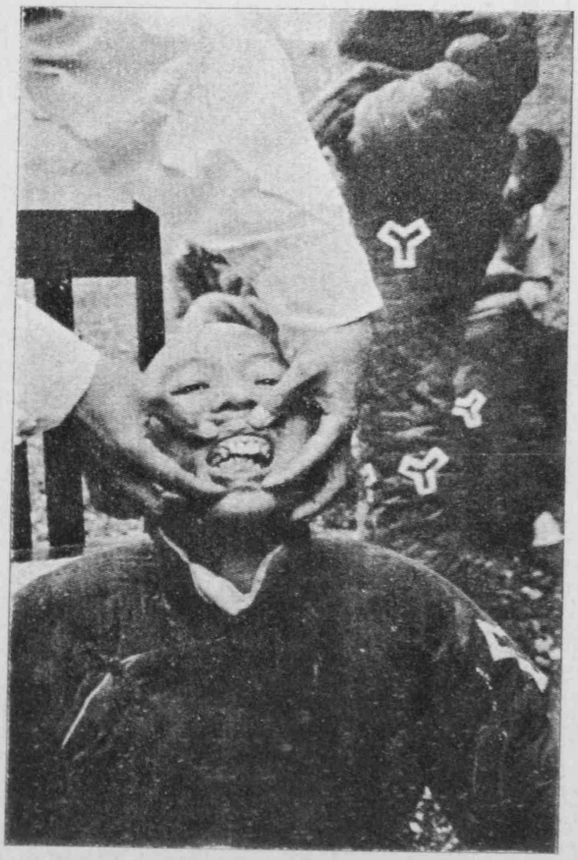
It has been found that one injection of Redoxon (Roche) given for four successive days or one orange and 6 oz. of raw turnips daily for nine days may arrest the disease and effect a cure if the pre-scurvy diet is changed.

I has been noticed that the working convicts are more prone to the attack than the ordinary ones although the former are allowed a more liberal diet as a rule.

The clinical picture is mainly that of budding of the gums which ooze easily and the development of petechial haemorrhages usually in the lower limbs. Sometimes multiple nodular swellings are found in the limbs not quite unlike that of erythema nodosum. One case has a deep haematoma in the thigh which has caused considerable difficulty in the diagnosis until other manifestations are evident. There is general prostration in some of the more advanced cases and slight oedema of the affected limb is not uncommon. In severe cases a positive Rumpbell-Reed's sign is readily obtainable. The blood picture of 15 cases studied shows secondary anaemia with a relative increase of lymphocytes. Bleeding into the joint or other serous cavities is probably extremely rare.

CASE No. 1648.

(I) Moderately swollem gums. 3.11.37.



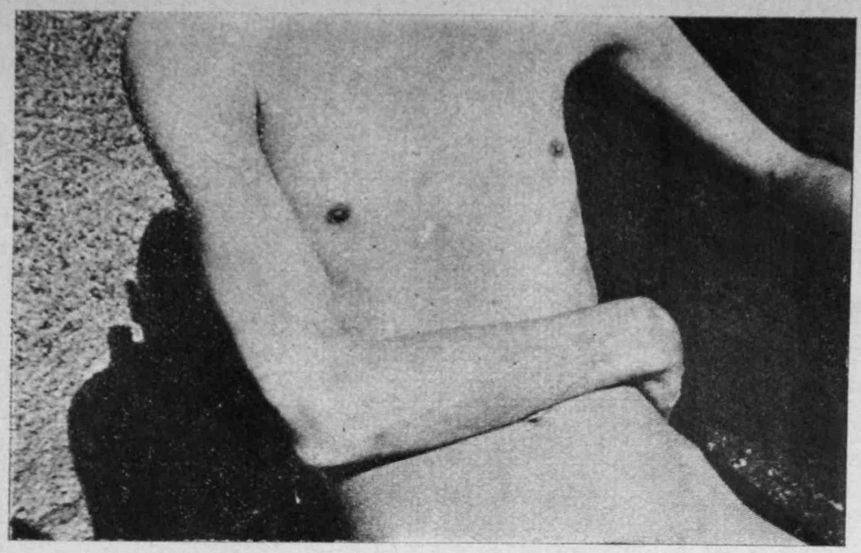
(12 days later)

(II) Gums became normal.
Had 4 injections of Redoxon.
1 each on 4th, 5th, 6th & 7th Nov. 1937.

CASE No. 1268.

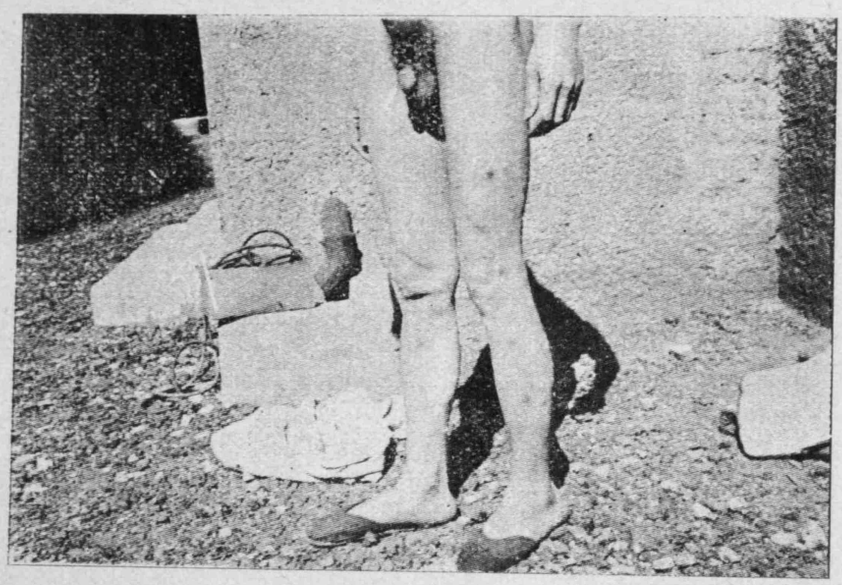
(III) Very markedly swollen gums which bleed readily. 28.10.37.

(IV) After 9 days' treatment with 1 orange and 6 oz. of turnips daily.



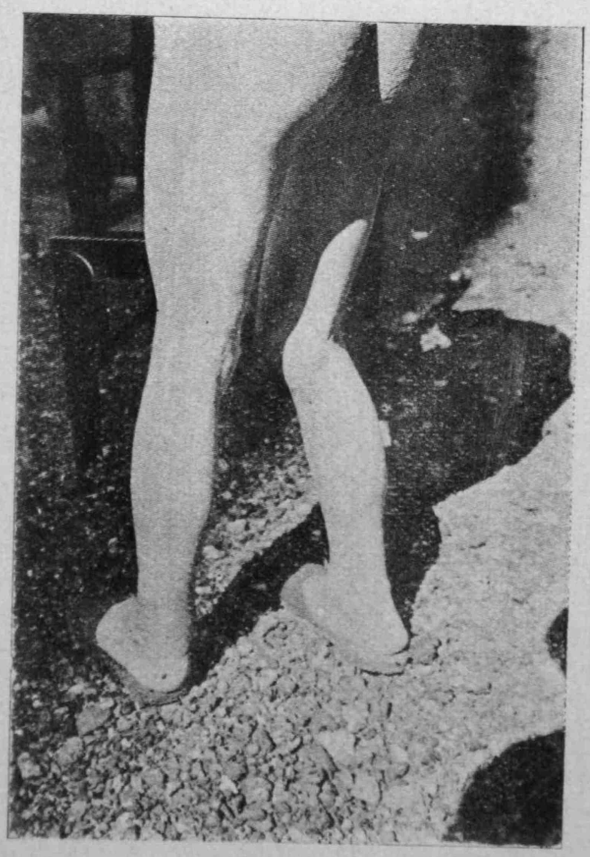
CASE No. 864.

(V) After Rumpbell-Reed's test.
Note the Petechial haemorrhage of the dorsal aspect of right forearm.



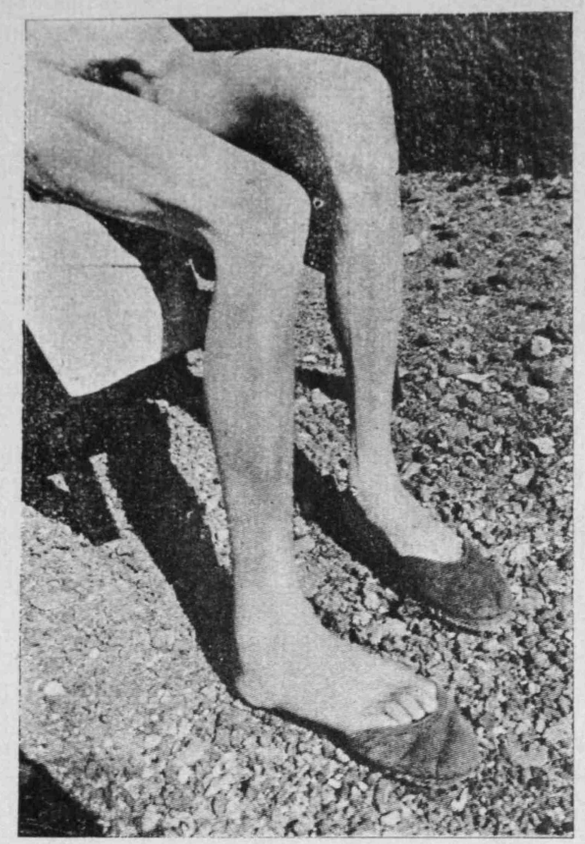
CASE No. 3140.

(VI) Subcutaneous haematomas resembling erythema nodosum.



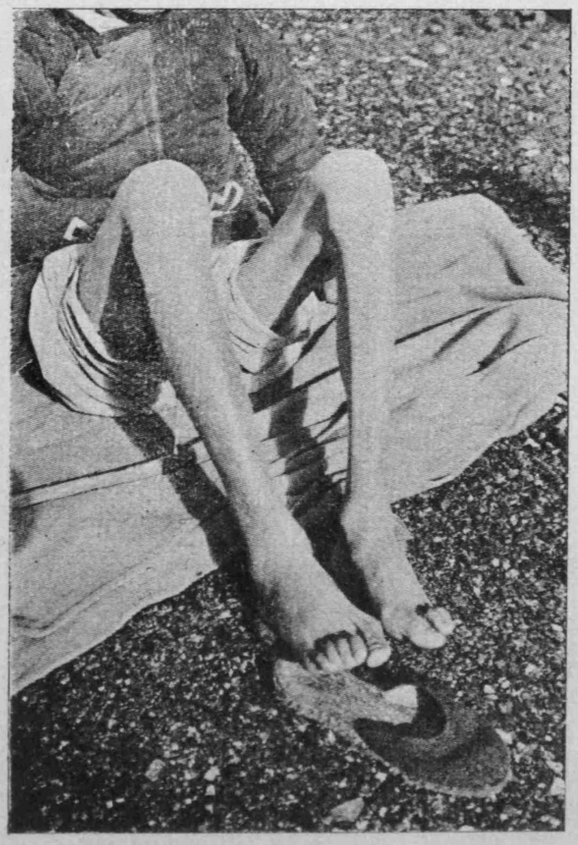
CASE No. 3768.

(VII) Discolouration and swelling of left ankle.



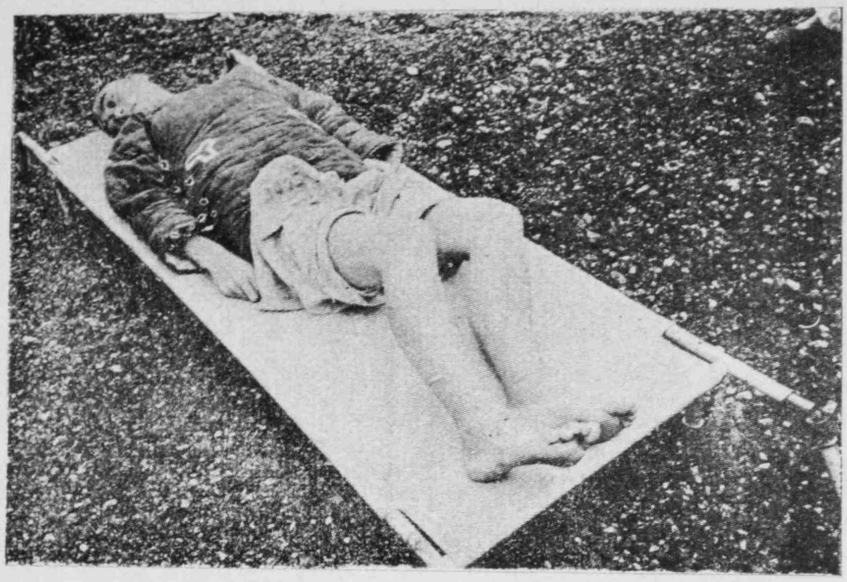
CASE No. 371.

(XIII) Right leg much swollen with large patch of discolouration. 28.10.37.



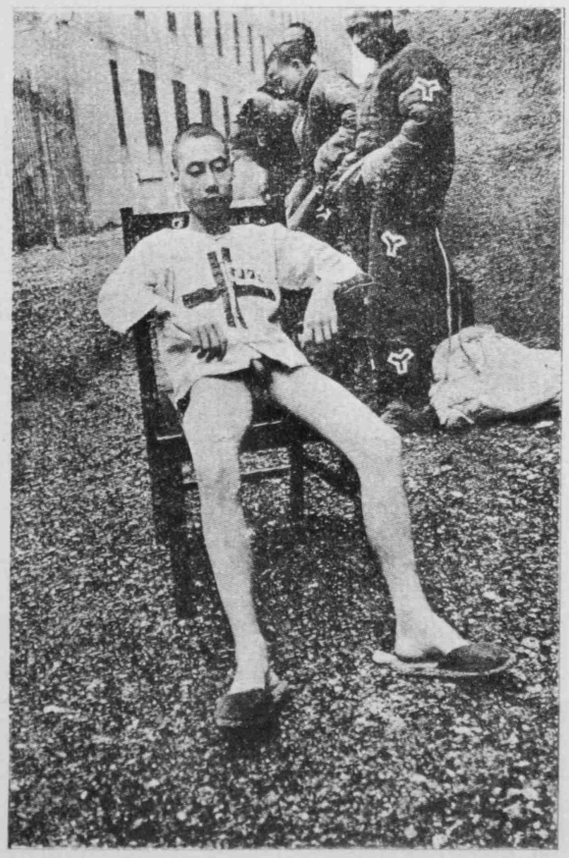
(IX) After 9 days' treatment with one orange and 6 oz. of raw turnips daily.

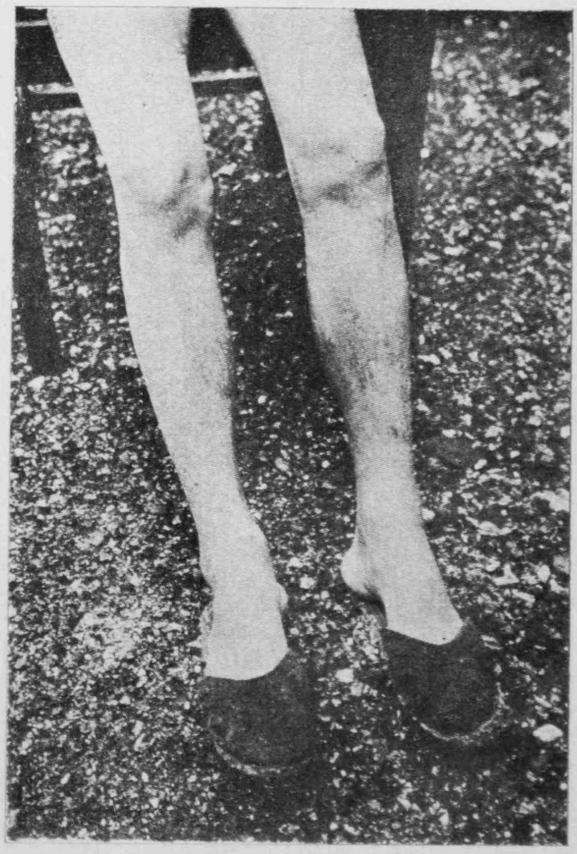
Discolouration disappearing and leg approaching normal.



CASE No. 3475.

(X) Markedly swollen legs with general prostration. 3.11.37.





(12 days later)

(XI) & (XII) After 4 injections of Redoxon. 1 each on 4th, 5th, 6th & 7th Nov., 1937.

General improvement.

Legs became normal in size.

No pitting on pressure.

Review of Books

Present-day medicine and pharmaceutical research:

Some observations on the "Bayer" Jubilee Year 1938.

Few branches of human activity to-day are so closely connected as medical science and pharmaceutical research. Both have the same object, the relief of suffering and are essentially dependent on one another in its attainment. The modern practitioner would often find himself helpless without modern drugs and the work of the investigator in the laboratory would be equally pointless without the practical collaboration of experienced doctors. The results achieved by this collaboration are remarkable in the extreme; modern medicine in company with chemico-pharmaceutical research has reached a standard undreamt of a generation ago. How would humanity fare without it? A survey of the last fifty years reveals the amazing advances that have been accomplished. Statistics show that human beings to-day live appreciably longer than their ancestors and that death occurs far more often than formerly from old age or accident, i.e. not from disease. This is largely due, apart from the general change in condition of living, to the skill of the modern doctor and the high standard of present-day pharmaceutical research.

The beginnings of the latter date from the eighties of the last century. Among the firms engaged in it " Bayer " 's may be said to occupy a special position. The discovery in the " Bayer " laboratoreis of the first antipyretic and the first analgesic marked an early success in the exploration of this new field of science, and the careful research and pioneer achievements that followed opened up many new aspects of therapeutics, for instance in chemotherapy, hormone and vitamin research, analgesics and tropical medicine.

The first " Bayer " preparation was evolved fifty years ago in a modest laboratory in Elberfeld; to-day there are few diseases for which the firm does not supply an effective remedy. The reputation these remedies have acquired has been built up by accurate scientific work in the laboratories where they are produced and by the close collaboration of the " Bayer " research workers with medical science and practice.

The world wide responsibilities of " Bayer" 's are well illustrated by the private air-liner, the Ju " Bayer," which the firm uses for the rapid transport of drugs and vaccines to threatened areas in cases of emergency and epidemics.

Many a traveller who passes Leverkusen by rail, road or Rhine steamer and sees the great electric sign, the " Bayer-Cross " towering over the works, will remember the small tablets, stamped with the " Bayer-Cross," which he once took himself. Every one of these

tablets, and likewise every ampoule, embodies the exact and conscientious collaboration of medical and pharmaceutical science which is typified in the "Bayer" works.

Aids to Embryology (Third Edition), by R. H. Hunter, *Baillière, Tindall & Cox.* Price 3/6 net.

This little book contains 178 pages of which 6 are index. In 172 pages Dr. Richard Hunter has contrived to compress a very useful summary of Embryology to suit the needs of the medical student.

The book opens with an account of the sex cells, their formation, maturation and fertilization. Then follows a very informative chapter on the transmission of hereditary characters, which includes a review of Mendelism and of more recent knowledge of Genetics in relation to blood groups, haemophilia and other condition.

The formation of the germ layers, of the foetal membranes and an outline of modern knowledge of ovulation relative to the menstrual cycle and the pituitary hormones follows in two short chapters.

The chapter on the skin gives a fair idea of the author's method. Following a paragraph on the development of the germ layers come in turn accounts of the development of the skin itself, of the nails, the hair, of the glands of the skin, sebaceous, sudoriferous and mammary, and finally a paragraph on Anomalies of Development.

Throughout the book we find after an account of the development of some organs or system of organs this same welcome feature—a paragraph on the Anomalies of Development. This paragraph on anomalies is not always at the end of a chapter; the end is often devoted to a concise summary of salient facts, but it is always present and by its presence widens the value of this book, by making it a useful place of reference for the advanced student.

The chapter on the nervous system covers 22 pages but presents to the student most of the important points in the development, as well as a summary and explanation of the congenital abnormalities. The chapters devoted to the development of the eye and of the ear are excellent.

The development of the alimentary canal and its associated glands occupies 26 pages, the anomalies of development would make a very formidable list if placed in continuity. The author has preferred to describe the abnormalities rather on a regional basis, e.g., the abnormalities of the pharyngeal region, of the intestinal tract, of the liver and pancreas, and so on.

The development of the circulatory system is quite full and includes an account not only of the septation of the heart and of the

development of the great arterial trunks but also of limb arteries and of the permanent residua of the various segmental anastomosing systems. The development of the venous system including the inferior vena cava and the portal vein is adequately reviewed. Anomalies of development are summarised.

Chapters are devoted to the development of the coelomic cavities and of the urogenital system in both sexes with short accounts of anomalies.

Even the muscular and skeletal systems receive attention in a single chapter. An appendix contains useful memoranda for the estimation of foetal age judged by size, weight and the degree of development of various structures.

This little book is adequately if not lavishly illustrated by clear line diagrams which immensely help the reader.

The teacher will be able to recommend the use of the "*Aids to Embryology*" not only to students contemplating entry for their examination in Anatomy and Physiology, but also to students in the various stages of clinical and post graduate study. In short the "*Aids to Embryology*" may be stated to justify the aims of its author and its title as a very useful aid to the study of Embryology at any stage of the students' career.

Aids to Histology (Fourth Edition), by A. Goodall, *Bailliere, Tindall & Cox*. Price 3/6 net.

The chief usefulness of this little book will be found in the "revision" stage of the students' course. A great deal of very useful information is compressed within the compass of 151 pages, which includes 15 pages of index.

This book is in no way to be recommended as a substitute in any sense for a larger work on Histology. The descriptions of microscopic structure are short and concise but the reader often gets the impression that the use of figures in the text has been rather ruthlessly cut down.

The Publishers claim that this book is in no sense a "cram-book." The teacher will probably agree with this view; the "*Aids to Histology*" is too short to cover the subject adequately, its purpose is to provide a series of very useful "aids" or reminders to students who have already made a systematic study of Histology.

If the "*Aids to Histology*" were inter-leaved with blank pages by the publishers (or by the student himself) to give room for amplification by the student of the diagrams which are reduced to a minimum, its usefulness might be greatly enhanced.

The chapters and the paragraphs seem somewhat uneven in their value. The opening chapter on the cell structure and cell division contains much useful information. The chapters on nerve and blood are useful but the paragraph on bone marrow is far too short and is included in the chapter on lymphatic tissues and the ductless glands. The chapter on the urinary system is too short to be really useful, even to convey brief reminders of the details of a complicated system. The central nervous system, with cranial nerves and organs of special sense, accounts for 37 out of the 151 pages, and probably is the most useful part of the book.

Thirteen pages are devoted to Histological methods and technique. Possibly many readers would prefer to see these pages used in amplification of chapters where information has been presented rather too shortly and where figures are altogether too few.

The "*Aids to Histology*" contains a great deal of information which will be useful to the medical student in revision of the subject and in anticipation of examination but can only be recommended on conclusion of a full laboratory and lecture course in Histology.

Revista de la Facultad de Medicina. Vol. 6. Nos. 10/12. 1938.

Revue Medicale Francaise D'Extreme-Orient. Vol. 16. Nos. 4/5. 1938.

Mitteilungen aus der Medizinischen Akadamie zu Kioto. Vol. 23. No. 4. 1938.

Taiwan Igakkai Zasshi. Vol. 37. No. 8. 1938.

Chiba Igakkai Zasshi. Vol. 16. Nos. 6/10. 1938.

Universidad Nacional de la Plata. Sections 1 & 2. 1937 & 1938.

The Chinese Medical Journal. Vol. 54. No. 3. 1938.

Unitas. Vol. 17. No. 2. 1938.

The Journal of the Shanghai Science Institute. Vol. 3. Sec. IV. Separate prints Nos. 16/17. pp. 239/268.

The Broadway. Vol. R7. No. 4. 1938.

University College Hospital Gazette. Vol. 23. Nos. 4/5. 1938.

Post Graduate Medical Journal. Vol. 14. No. 155. 1938.

Journal of the Malaya Branch of the British Medical Association. Vol. 2. No. 2. 1938.

Arquivo de Patalogia. Vol. 9. Nos. 2 & 3, 1937. Vol. 10. No. 1. 1938.

Bulletin de la Facultad de Medicine d'Istanbul. Vol. 1. No. 3. 1938.

Fukuoka Acta Medica. Vol. 31. Nos. 9/10. 1938.

The New Zealand Medical Journal. Vol. 37. Nos. 200/1. 1938.

Intercambio Universitario. 1937.

The Hospital. Vol. 34. Nos. 9/11. 1938.

Okayama Igakkai Zasshi. Vol. 50. Nos. 8/10. 1938.

The Tohoku Journal of Experimental Medicine. Vol. 34. Nos. 1/5. 1938.

Anales le la Facultad de Ciencias Medicas. Vol. 20. No. 3. 1938. Vol. 21. No. 1. 1938.

Collected Papers from the Faculty of Medicine. Osaka Imperial University. 1937.

Review Medico-Chirurgicale. Vol. 48. Nos. 4/6. 1937.

Bulletins from the Institute for Melical Research Federated Malay States. Nos. 1 & 2, 1937. No. 1. 1938.

Annual Report of the Institute for Medical Research. Federated Malay States. 1937.

Oriental Journal of Diseases of Infants. Vol. 23. No. 3. 1938. Vol. 24. No. 1. 1938.

Boletin Sanitario. Supplement. No. 2. 1938. Vol. 2. Nos. 5/7. 1938.

Arbeiten aus der Medizinischen Fakultat Okayama. Vol. 6. No. 1. 1938.

Bulletin of the New York Academy of Medicine. Sept./Oct., 1938.

Mededeelingen van den Dienst der Volksgezondheid in Nederlandsch-Indie. Vol. 27. No. 4. 1938 and Supplement.

Universidad de la Habana. Vol. 3. No. 19. 1938.

The Journal of Bone and Joint Surgery. Vol. 20. No. 4. 1938.

Middlesex Hospital Journal. Vol. 38. No. 5. 1938.

Medicina de Hoy. Vol. 3. Nos. 4/8. 1938.

Bulletin of the Health Organisation of the League of Nations. Vol. 7. Extract No. 9. 1938.

King's College Hospital Gazette. Vol. 17. No. 4. 1938.

Medical Gazette (Dr. Sun Yat Sen Medical College). Vol. 1. No. 1. 1938.

Helvetica Medica Acta. Vol. 5. No. 4. 1938.

The Ulster Medical Journal. Vol. 7. No. 4. 1938.

Health and Empire. Vol. 13. No. 3. 1938.

Epidemiological Report. Vol. 17. No. 8. 1938.

The Nagoya Journal of Medical Science. Vol. 12. No. 3. 1938.

Archives of Medical Hydrology. Vol. 16. No. 4. 1938.

St. Mary's Hospital Gazette. Vol. 44. No. 8. 1938.