they are now permanently avirulent for man and are consequently of no epidemiological significance.

Fluctuations undoubtedly occur in the virulence and invasiveness of the meningococci. Maclean and Bevan (1939), investigating an extensive outbreak in Cyprus, obtained clinical evidence that suggested an increase in virulence of the meningococcus as a result of frequent passage from man to man. They recorded many instances of the rapid transfer of catarrh inside family circles, while only the last victims of the common infection developed meningitis. They did not, however, obtain any bacteriological confirmation of these observations.

It is consequently impossible to give any final opinion about the virulence of Group 1 and Group 2 meningococci, but there is no doubt that at the present time Group 2 strains are relatively avirulent and have a low invasive power. It is possible that changes may take place in meningococci analogous to those found in *H. pertussis*, where virulence progressively decreases from Phase I to Phase IV. It is interesting to note that while Group 1 strains isolated from cases of cerebrospinal meningitis usually gave clear-cut agglutination with little cross-reaction with Group 2 sera, strains isolated from carriers (mainly Group 2) frequently exhibited marked overlapping with the two group sera. Strains isolated from actual cases tended to show greater group specificity than strains isolated from carriers.

The spread of the meningococcus in the community is by droplet infection, and such factors as poor ventilation and overcrowding undoubtedly play an important part in the dissemination of the organism. They do not, however, appear to play a primary part in the development of the disease. Several of the 51 cases here recorded were associated with serious overcrowding; yet in no instance did a second case of cerebrospinal meningitis develop, in spite of the fact that such cases had often not been isolated for some twenty-four hours after the onset of the disease. A carrier rate of 21% was, moreover, found in the patients of the hospital; these were living under ideal conditions, with good ventilation and no overcrowding, the distance between adjacent beds being at least four feet. The organisms isolated, however, generally proved to be the relatively avirulent Group 2 strains.

During this investigation it has been repeatedly found that fatigue has been a contributing factor in the development of the disease. It is a common experience that the disease is found most commonly in young recruits and, under wartime conditions, vigorous training is started early, often in individuals unaccustomed to violent exercise. As a result resistance to infection is lowered rather than increased. The importance of overcrowding as a factor in the development of cerebrospinal meningitis appears to have been exaggerated, to the neglect of such factors as fatigue, which tend to lower resistance to infection.

The meningococcus is widely distributed in the community and, although the organism does not live naturally outside human tissues, its complete elimination is impossible. Swabbing is consequently of little value except in special circumstances, such as when a persistent source of infection is present in a closed community or during an extensive localized outbreak, where all contacts can be swabbed and carriers segregated and treated. These points have been stressed in the recent War Office Memorandum on Cerebrospinal Fever (1940).

The use of sulphonamide drugs in the treatment of carriers is likely to have only a limited application, as their wholesale administration is impossible. When, however, swabbing has been carried out and carriers have been identified these drugs should prove of great value, since

other methods of treating carriers have proved unreliable. The results recorded here indicate that in adequate dosage the administration of sulphapyridine is an effective and rapid method of removing meningococci from the nasopharyngeal mucosa.

Summary

Fifty-one cases of cerebrospinal meningitis have been investigated; the meningococcus has been isolated on twenty-three occasions and, where typed, has been Group 1.

Evidence has been obtained that adequate dosage with sulphapyridine is a satisfactory method of clearing meningococci from the nasopharyngeal mucosa.

I wish to thank the many persons—in particular Mr. C. W. Ashton, senior laboratory assistant—who have assisted in various ways during this investigation.

REFERENCES

- Banks, H. S. (1939). *Lancet*, **2**, 921.
 Dudley, S. F., and Brennan, J. R. (1934). *J. Hyg., Camb.*, **34**, 525.
 Emberton, D., and Steven, G. H. (1919). *Lancet*, **1**, 788.
 Glover, J. A. (1918). *J. Hyg., Camb.*, **17**, 367.
 — (1920). *Med. Res. Cncl. Spec. Rep. Ser. No. 50*, p. 133, London.
 Maclean, I. H., and Bevan, C. E. (1939). *Proc. roy. Soc. Med.*, **32**, 1551.
 Sandiford, B. R. (1937). *J. Path. Bact.*, **45**, 467.
 War Office (1940). *Memorandum on Cerebrospinal Fever among Troops*, London.
 Wilson, S. P. (1928). *J. Path. Bact.*, **31**, 477.

ANALYSIS OF HAEMORRHAGIC STATES WITH SNAKE VENOM AND LECITHIN

BY

L. J. WITTS, M.D., F.R.C.P.

Nuffield Professor of Clinical Medicine

AND

FELICITY C. G. HOBSON, B.A.

(From the Radcliffe Infirmary, Oxford)

In numerous experiments in man and animals we have confirmed the observations of Trevan and Macfarlane (1936-7) that the coagulant action of Russell-viper venom on blood and plasma can be potentiated by the addition of lecithin. Leathes and Mellanby (1939) and Pratt (1940), working with solutions of prothrombin, have shown that the venom acts as a thrombokinase, converting prothrombin into thrombin in the presence of calcium, and they have clearly demonstrated the adjuvant action of lecithin.

Method Used

All the observations in this paper were made by substituting venom or venom plus lecithin for the brain extract used in Quick's prothrombin test (Quick, 1935):

Nine cubic centimetres of blood, withdrawn rapidly and with special precaution to avoid trauma, are promptly and thoroughly mixed with 1 c.cm. of M/10 sodium oxalate, and centrifuged at a low speed for five minutes. Of this plasma 0.1 c.cm. is transferred to a dry clean test-tube (13 × 100 mm.) in a water bath kept at 37° C., and mixed with 0.1 c.cm. of venom or venom plus lecithin. Without delay 0.1 c.cm. of M/40 calcium chloride is added, the tube quickly shaken, and the exact time required for the formation of a solid clot recorded.

Under the conditions of Quick's test the optimum amount of lecithin is about 5 mg. per c.cm. of venom (Hobson and Wits, 1940), and the figure remains very constant under various experimental conditions, in different animal species, and in different members of the same species. This concentration is obtained by adding 0.05 c.cm. of a 10% alcoholic solution of ovo-lecithin to every cubic centimetre of the solution of venom. Different batches of Russell-viper venom as purchased vary in coagulant power, but phials from the same batch are very constant.

The Investigation

Our observations were made with a single batch of venom, which was used in a dilution of 1 in 50,000—i.e., 0.1 mg. venom + 1 c.cm. of distilled water + 4 c.cm. of saline. The plasmas were from a random group of 43 normal and sick people, with a bias in favour of cases of haemorrhagic disease. In each case we measured the accelerated clotting time using venom alone and venom plus lecithin (see Table and Fig. 1).

Table of Cases, showing the Coagulation Time of Plasma in Seconds by Various Techniques

Sex and Age	Diagnosis	1/50,000 Venom	Venom + 5 mg. per c.cm. Lecithin	Lecithin Differ- ence	Quick	Howell
M. 27	Normal	sec. 20	sec. 10	sec. 10	sec. 19	sec. 110
M. 26		24	11	13	19	90
M. 58	Arthritis	22	12	10	—	—
M. 43	"	20	11	9	17	127
M. 51	Sciatica	20	9	11	—	—
M. 38	"	23	11	12	—	—
M. 36	Tuberculous meningitis	19	13	6	16	—
M. 52	Pulmonary osteo-artro- pathy	21	14	7	16	—
M. 63	Gastric ulcer	24	11	13	19	120
M. 25	Convalescent burns	20	11	9	—	—
F. 56	Epistaxis	20	11	9	19	80
F. 17	Ulcerative colitis	22	12	10	—	—
F. 49	Hypertension	15	11	4	16	—
M. 36	Traumatic injury of heart	13	10	3	—	—
F. 64	Coronary thrombosis	25	13	12	—	—
M. 53	Diabetes mellitus	18	12	6	—	—
M. 66	Prostatic hypertrophy	—	10	—	—	—
M. 39	Thyroidectomy	21	11	10	—	—
F. 29	Dysmenorrhoea	20	11	9	19	140
M. 59	Acromegaly	25	11	14	16	—
M. 40	Subacute combined degeneration	28	13	15	—	—
F. 70	Myeloclerosis	19	11	8	18	80
F. 58	Aplastic anaemia	25	12	13	—	—
F. 40	Nocturnal haemoglobinuria	20	9	11	—	—
F. 68	Pernicious anaemia	20	11	9	19	120
M. 60	Cirrhosis hepatis	—	14	—	—	—
F. 64	Obstructive jaundice	—	14	—	—	—
M. 45	"	24	14	10	29	110
F. 62	Treated jaundice	14	12	2	20	180
M. 5	Haemophilia	21	11	10	16	240
M. 2	"	21	12	9	23	300
M. 6	"	20	10	10	19	420
M. 37	"	17	12	5	19	140
M. 1	"	19	12	7	18	165
F. 29	Carrier of haemophilia	18	12	6	20	180
M. 5	Thrombocytopenia	22	11	11	19	180
M. 12	Fibrinopenia	25	11	14	20	195
F. 30	Thrombocytopenia	29	10	19	18	120
M. 12	"	28	13	15	—	—
The Den Family:						
M. 25	Albert (thrombasthenia)	29	12	17	—	—
M. 31	Arthur	29	12	17	17	140
F. 10	Joan	21	15	6	18	105
M. 5	Billy (no symptoms)	20	11	9	19	90

FIG. 1.—Accelerated clotting time with venom alone and venom plus lecithin.

The Den Family.—Affected members bleed readily from cuts and dental extractions and frequently from the nose. Coagulation time (Lee and White) is normal, bleeding time sometimes prolonged, platelets low normal, clot retraction indifferent, and fibrinogen normal.

Fibrinopenia.—This case—that of a boy aged 12—has presented symptoms very much like haemophilia, but the clotting time (Lee and White) is normal, as are the bleeding time and the platelets; clot retraction is poor and fibrinogen 142 mg. per 100 c.cm.

FIG. 2.—Pedigree of the Den family.

With venom alone the most frequent clotting time was 20 seconds; but the observations were widely scattered, and unusually long clotting times were noted in patients in whom Quick's test gave a normal result.

With venom plus lecithin clotting times were not only quicker but much less scattered. The usual time was 11 seconds. The frequency curve is of a bimodal type, and suggests a normal range of 9 to 13 seconds. The plasmas which gave values over 13 seconds came from cases of cirrhosis (14"), obstructive jaundice (14" and 14"), thrombasthenia (15"), and pulmonary osteo-artro-pathy (14"). High values were expected in disease of the liver, in which the amount of prothrombin in the plasma is reduced and the clotting times with Quick's reagent and venom plus lecithin run parallel. The high values in thrombasthenia and pulmonary osteo-artro-pathy, which did not check with Quick's test, were only appreciated in retrospect and have not yet been explained.

The interval between the peaks of the two curves is 9 seconds. We call this difference between the clotting times with venom alone and venom plus lecithin the *lecithin difference*, as it is a measure of the adjuvant action of lecithin. The values for the lecithin difference are very scattered, and they obviously account for the irregularity of the results when venom alone is used. The usual lecithin difference is 9 or 10 seconds, but the figures range from 2 to 19 seconds. Our data are not sufficient to enable us to find rhyme or reason for small lecithin differences, but of the seven lecithin differences above 13 seconds two are in thrombocytopenic purpuras, two in a thrombasthenic family, one in a congenital haemorrhagic diathesis we have labelled "fibrinopenia," one in acromegaly, and one in subacute combined degeneration. The only other thrombocytopenia we have examined—not included in this series because only 2 mg. per c.cm. of lecithin was used—had a lecithin difference of 18 seconds. In our brief experience a high lecithin difference is not always found in a given case of haemorrhagic diathesis, and, like so many other phenomena in that puzzling group of diseases, it seems to vary with the intensity of the disorder.

Conclusion

Observations on the use of Russell-viper venom alone and venom plus lecithin as the clot-accelerating reagents in Quick's test suggest that this technique allows us to recognize three types of haemorrhagic tendency. In haemophilia the blood coagulates like that of normal people when snake venom is added. In purpura haemor-

rhagica and certain hereditary haemorrhagic states coagulation may be less rapid than normal when venom alone is used, but is not usually different from normal when venom and lecithin are added; the need for lecithin varies at different periods and may run parallel to the tendency to bleed. In prothrombin deficiency coagulation remains impaired despite the addition of venom plus lecithin. If venom is used instead of brain extract to measure prothrombin in the Quick test it must be fortified by the addition of lecithin; in other words, *venom (thrombokinase) + lecithin = tissue extract (thromboplastin)*.

REFERENCES

- Hobson, F. C. G., and Witts, L. J. (1940). *Lancet*, **2**, 247.
 Leathes, J. B., and Mellanby, J. (1939). *J. Physiol.*, **96**, 39 P.
 Pratt, C. L. G. (1940). *Ibid.*, **98**, 20 P.
 Quick, A. J. (1935). *J. biol. Chem.*, **109**, lxxiii.
 Trevan, J. W., and Macfarlane, R. G. (1936-7). Report of Medical Research Council, p. 143.

POLYCYTHAEMIA AND VITAMIN C

BY

JAMES DEENY, M.D., M.Sc., D.P.H., M.R.C.P.I.

Lurgan

For several years past it has been considered that vitamin C plays some part in the regulation of the number of red blood cells in the body. Dunlop and Scarborough (1935) showed that a rise in the red blood count, a decrease in the number of reticulocytes, and a rise in the haemoglobin percentage resulted when vitamin C was given to a patient suffering from scurvy, although the iron content of the diet (10 mg. daily) was not increased. On the other hand, Barron and Barron (1936) observed that vitamin C reduced the red blood count in animals suffering from artificially induced polycythaemia. Waltner and Waltner (1929) noted that this disease could be caused in animals by administering cobalt, which Mascherpa (1930) was able to demonstrate caused an increase in bone marrow activity in dogs. When they simultaneously injected cobalt sulphate and vitamin C intravenously (60 mg. daily) into rabbits Barron and Barron failed to observe any signs of polycythaemia, although this appeared a week after withdrawing the vitamin C.

When vitamin C was injected after the production of polycythaemia a decrease in the red cell count and the haemoglobin percentage resulted, although the effect was temporary. It was found that the percentage of haemoglobin ran parallel with the red blood count. This experimental polycythaemia is probably caused by the cobalt inhibiting the respiratory function of immature red blood cells, which are rapidly replaced in the bone marrow. Vitamin C functions as a regulator of the level of red cells in the circulating blood.

Barron and Barron (1936) produced polycythaemia in rabbits by the daily injection of 0.01 gramme of cobalt sulphate. Reticulocytes and erythroblasts appeared in the circulating blood and there was an increase in respiration of the red blood cells, although the latter was not observed in cases of human polycythaemia vera. The addition of cobalt sulphate to red cell suspensions from the blood of rabbits suffering from cobalt polycythaemia was followed by marked inhibition of respiration, in contrast with the absence of such an effect in similar suspensions prepared from the blood of normal rabbits. This inhibition of respiration was confined to immature red cells, being greater in the bone marrow cells than in the red cells of the kidney and spleen.

According to Barron one of the functions of vitamin C is to maintain the haemoglobin concentration in the blood

at a normal level. This view is based on the following facts: (1) The fall in the percentage of haemoglobin in the early stages of scurvy occurs before clinical symptoms appear, and the haemoglobin returns to normal after the administration of vitamin C, before these symptoms disappear. (2) Cobalt polycythaemia in rabbits is prevented by giving vitamin C. It is also known that vitamin C reduces ferrihaemin to ferrohaemin, which when combined with globin yields haemoglobin. There is some evidence that the utilization of iron is defective when the reserves of vitamin C in the body are depleted (McCollum *et al.*, 1939). Neither large doses of iron nor the substance potent in pernicious anaemia are effective in the scorbutic state.

Kandel and LeRoy (1938) tried the effect of vitamin C on two patients suffering from polycythaemia vera, but reported that it had no influence whatsoever on the red blood count and the haemoglobin percentage.

Encouraged by Barron and Barron's results a study has been made of the vitamin C metabolism in two cases of polycythaemia. I decided to investigate the excretion of the vitamin, the amount necessary to saturate the patient, the result of giving large doses, and finally the effect of raising the threshold of vitamin C excretion by administering sodium bicarbonate.

Hawley *et al.* (1936) showed that the amount of vitamin C retained by the body depends upon factors other than the level of the vitamin already in the body. For example, when vitamin C is administered with ammonium chloride to persons already saturated with the vitamin almost 100% of a daily test dose is excreted. If, however, the same amount of vitamin C is given with sodium bicarbonate only 50% is excreted in the urine. Similar experiments with guinea-pigs showed that the administration of large doses of sodium bicarbonate permitted the storage of greater amounts of vitamin C in the adrenals and liver than when the vitamin was given alone or with ammonium chloride.

Dr. J. G. Keohane of Limavady, who had a case of polycythaemia under his care, carried out a similar investigation at my request.

Case Records before starting the Investigation

Case 1.—A fisherman aged 53. This patient was a strong well-built man whose only illness had been pneumonia ten years ago. About two years ago he began to feel a heaviness in the head, and suffered from headaches, lack of concentration, and vertigo. He had pains in his legs and was easily tired. His face became a deep purple colour, particularly so in the lips and cheeks. His eyeballs were bloodshot and the conjunctivae congested. He was admitted to the Royal Victoria Hospital on April 12, 1939, under Prof. W. W. D. Thompson. He then complained of pains in the head and chest and heaviness in the head. His complexion had become more purple. On examination his lips and the conjunctival surfaces of the eyelids were a deep crimson. The conjunctivae were injected and the skin of his body was redder than normal. His apex beat was in the sixth intercostal space, 4 inches from the midline. The blood pressure was 148/95. There were some crepitations at the base of the right lung. The temperature was normal.

The patient had an unhealthy mouth and his abdomen was distended. Palpation revealed a sausage-shaped tumour 4 by 1½ inches in the right iliac fossa. Neither liver nor spleen was palpable. The results of a blood examination were as follows: red cells, 6,290,000 per c.mm.; Hb (Haldane), 106%; colour index, 0.9; white cells, 10,000 per c.mm.; Wassermann negative. Nothing abnormal was found in the cerebrospinal fluid or urine. Cystoscopy and a barium enema also revealed nothing abnormal.

A diagnosis of polycythaemia was made. The patient was advised laparotomy, but refused. On April 19 venesection was performed and 500 c.cm. of blood withdrawn. Another