during our six-day observation, but throat washings from four subjects were positive. One healthy subject developed clinical illness 12 hours after blood and throat-washing specimens were obtained. These were positive for influenza, and the blood isolates were sent to the World Influenza Centre, being confirmed by Dr. Pereira to be the Hong Kong variant. Reisolation of the virus from the original blood specimen was successful, but no virus was detected from the blood specimens obtained 12 and 24 hours after clinical manifestation. The paired sera of this case showed a 16-fold rise both to the blood isolate and to the reference antigen. Table II lists viral isolation and haemagglutination inhibition antibody of healthy contacts, with positive isolations.

TABLE II.—*Antibody Titres to Asian Influenza (Hong Kong variant) in 5 Healthy Contacts with Positive Virus*

Contact's No.	Viral Isolation		Reference Antigen		Isolate	
	Throat	Blood	Acute	Con- valescent	Acute	Con- valescent
1*	+	+	1:8	1:256	<1:8	1:128
2	+	—	1:16	N.T.	<1:8	N.T.
3	+	—	1:1,024	1:128	1:512	1:64
4	+	—	1:1,024	N.T.	1:512	N.T.
5	+	—	1:32	N.T.	1:16	N.T.

* Developed clinical symptoms of influenza 12 hours after obtaining specimen.

Discussion

Recovery of influenza virus from extrapulmonary tissue of man and animals was the first indication of the occurrence of viraemia during influenza infection (Hamre *et al.*, 1956; Kaji *et al.*, 1959; Oseasohn *et al.*, 1959).

Recovery of influenza virus from a patient's blood with clinical manifestations of the disease was the first report of proved viraemia in man (Naficy, 1963). Several other investigators, however, had failed to demonstrate viraemia during the clinical course of influenza infection (Kilbourne, 1959; Minuse *et al.*, 1962; K. Naficy, unpublished data). Stanley and Jackson (1966), using human volunteers, showed clearly that viraemia occurs during the incubation period and that the virus was not detected after the third day of infection. Our results demonstrate that in 12 out of 21 patients with clinical signs of influenza virus was isolated from the throat-washing specimens but none from their blood; while in one patient—who proved to be in the incubation period at the time

specimens were obtained—virus was obtained from both the blood and the throat washings. These results are in agreement with Stanley and Jackson's report and clearly explain accounts of unsuccessful attempts to demonstrate viraemia during the symptomatic phase of influenza infection.

The first successful report of the isolation of influenza virus from human blood, however, remains unexplained, since the isolation was made while the patient was symptomatic. Nevertheless, a review of the history of this patient showed that there had been two phases of clinical symptoms: (1) before admission and during the first two days of hospitalization, after which he became almost asymptomatic; and (2) a second phase from the fifth day, when he again experienced fever and chills (Naficy, 1963). Thus it is conceivable that fever and chills on the fifth day of hospitalization marked the onset of his influenza, unrelated to his undetermined previous infection, and the specimens were obtained during the incubation period.

It should be noted that four healthy subjects from whom virus was isolated remained asymptomatic. Two of these had a high haemagglutination inhibition antibody titre (1:1,024) in their acute sera. Thus it seems that, in spite of high circulating antibody, local replication of the virus in the nasopharyngeal cavity takes place, and may play a part in spreading the infection.

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Medical Memoranda

Thrombotic Microangiopathy Associated with Squamous Carcinoma

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The association of malignant disease with thrombophlebitis migrans is well recognized, and in some instances the lesions of the veins may be the first indication of occult malignant disease (Sproul, 1938). Such patients may also have non-bacterial thrombotic endocarditis (MacDonald and Robbins, 1957). On the other hand, disseminated arteriolar and capillary lesions occur much less frequently and do not normally give rise to clinical manifestations (McKay and Wahle, 1955; Azzopardi, 1966). Recently we had the opportunity of studying a patient who presented with features of thrombotic

thrombocytopenic purpura, and only at necropsy did it become clear that these were associated with widespread thrombosis of small vessels and recurrent carcinoma.

CASE REPORT

The patient was 56 years old when she was first seen in December 1963 complaining of rectal bleeding. This proved to be due to a rather poorly differentiated squamous carcinoma situated in the anal canal. Metastatic squamous carcinoma was also found in inguinal lymph nodes removed in a block dissection seven months later. After this, however, she remained well for nearly five years until bleeding occurred from the colostomy in November 1968. When admitted to hospital, after having symptoms for three days, she was severely anaemic and had a thrombocytopenia (Hb 3.7 g./100 ml., white cells 9,000/cu. mm., and platelets 65,000/cu. mm.). Blood transfusion brought some improvement in the haemoglobin level. Six days after admission, however, the platelet count was still only

66,000/cu. mm., and a purpuric rash had appeared on the abdomen and legs. A blood film at this time showed some fragmentation of the red cells with helmet and burr cells. In view of the clinical findings and the blood picture a diagnosis of thrombotic thrombocytopenic purpura was made and treatment started with prednisolone 40 mg. daily. Further blood transfusions were also given. During the next few days, however, she developed thrombophlebitic lesions in peripheral vessels and both feet became gangrenous. Three days after starting the prednisolone she showed evidence of weakness of the right arm and leg and dysphasia. She remained in this state until she died a further four days later.

Necropsy Findings.—Externally there was a purpuric rash on the abdomen and lower limbs and extensive gangrene of both feet. Internally, lymph nodes measuring up to 1.5 cm. in diameter were present along the common iliac vessels and abdominal aorta. The heart showed patchy myocardial fibrosis and there were vegetations 1 cm. in diameter on both aortic and mitral valve cusps. Consolidation was present in the lower lobe of the right lung. Examination of the brain showed a small infarct in the anterior part of the internal capsule on the left side. Thrombus was present in a branch of the right renal artery, in the left internal carotid artery, in the external iliac and femoral arteries on both sides, and in a number of large veins in the pelvis.

Histological Findings.—Metastatic squamous carcinoma was present in the enlarged lymph nodes, in the wall of the bladder, and in the myometrium. Tumour was also seen inside small vessels in the lungs. There was no evidence of metastatic tumour in sections of bone marrow from the vertebral column, sternum, and long bones. The vegetations on the heart valves consisted largely of fibrinoid material, there being no signs of bacterial endocarditis. Sections from the lungs, myocardium, spleen, uterus, and thyroid contained many arterioles and capillaries whose lumen was partly or completely occluded by thrombus (see Fig.). In some vessels the

Thrombus partly occludes the lumen of both small vessels. (H. and E. $\times 200$.)

thrombus appeared to be located entirely within the lumen, while in the others it merged with areas of fibrinoid necrosis in the inner part of the vessel wall. Varying degrees of organization of the thrombi were seen.

COMMENT

The clinical picture of neurological signs, gastrointestinal haemorrhage, and purpura, together with thrombocytopenia and changes in the red blood cells, was in keeping with the diagnosis of thrombotic thrombocytopenic purpura. The necropsy findings of widespread thrombi in small vessels, associated in some places with areas of fibrinoid necrosis in the walls of the vessels, are also identical with those found in thrombotic thrombocytopenic purpura. Nevertheless, in this case metastatic carcinoma was also present, and there is no doubt that we were dealing with a thrombotic microangiopathy resulting from the presence of metastatic carcinoma. Fisher

and Baird (1956), who described two cases of carcinoma associated with lesions of small vessels, drew attention to the similarity of the clinical features in their patient who had carcinoma of the bronchus to those in thrombotic thrombocytopenic purpura. We, however, have been unable to find reports in the literature in which the clinical and haematological features were so prominent that they resulted in the diagnosis being made during life.

The interrelationships of the bleeding, the vascular lesions, the thrombocytopenia, and the metastatic tumour are still obscure. Intravascular coagulation occurs in patients with carcinoma, and might account for the presence of disseminated thrombi in vessels (Brain *et al.*, 1962). Utilization of platelets in this process could result in thrombocytopenia and haemorrhage. Intravascular coagulation, however, is more commonly associated with defibrination, and the platelet count is not as a rule seriously lowered (Azzopardi, 1966). Moreover, it has been suggested that the changes of the vessel wall may precede thrombosis in cases of thrombotic microangiopathy associated with carcinoma, some workers considering that these changes are allergic in nature and result in extrusion of the material into the lumen (Fisher and Baird, 1956). This is similar to the proposed pathogenesis of the vascular lesions in primary cases of thrombotic thrombocytopenic purpura (Gore, 1950; Moore and Schoenberg, 1960). In addition, it has been suggested that the reduction in platelet count may also be the result of a similar direct allergic reaction rather than a consequence of platelet utilization, for thrombocytopenia is not invariably present in cases of thrombotic microangiopathy associated with carcinoma (Fisher and Baird, 1956). The fragmentation of the red cells has been explained in the same way, though it has been shown that similar changes can be produced mechanically and may well result from passage of the cells along abnormally narrowed arterioles and capillaries (Bull *et al.*, 1968).

The more commonly found manifestations of non-bacterial thrombotic endocarditis and thrombosis of large vessels were also present in this case, but an unusual feature is the histological type of carcinoma concerned. The great majority of descriptions of thrombophlebitis migrans and non-bacterial thrombotic endocarditis have been in patients with adenocarcinoma (Azzopardi, 1966). A similar predominance of glandular tumours also appears in the few reports of carcinoma associated with thrombotic microangiopathy, though renal capillary thromboses were observed in one woman with a squamous cell carcinoma of the cervix (McKay *et al.*, 1955).

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