Summary

Tissue cultures were prepared from 100 amnions by a method which was found to be reasonably easy and which gave satisfactory results. Human-amnion cultures were shown to be capable of detecting the great majority of prototype strains of enteroviruses, and were as successful as monkey-kidney cultures in isolating enteroviruses, with the exception of Coxsackie B strains, from clinical specimens.

We are grateful to Miss M. Buchanan and to Mr. E. J. Stott for their assistance in preparing amnion cultures; to the labour ward sisters, Robroyston Hospital, Glasgow, for their unflinching co-operation in obtaining placentas for this work; to Dr. A. D. Macrae, Virus Reference Laboratory, Colindale, for prototype strains of virus; to Dr. N. R. Grist and Dr. C. A. C. Ross, of this laboratory, for the results of those of the chorio-allantoic membrane inoculations carried out by them; and to our clinical colleagues who submitted specimens from their patients.

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CONGENITAL LEUKAEMIA WITH PARENTAL CONSANGUINITY CASE REPORT WITH CHROMOSOME STUDIES

BY

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Congenital leukaemia with evidence of the disease present at birth is a rare occurrence. It presents problems of diagnosis and management to the clinician and pathologist, and is of particular interest to the cytogeneticist from the standpoint of aetiology.

We have recently had under our care an infant with congenital leukaemia whose parents were first cousins. The opportunity was taken to study the chromosomes of the infant and both parents, and it was possible to observe for a short time the effect of treatment with prednisolone.

Case Report

The infant, a male, was born on January 22, 1961, at Mill Road Maternity Hospital, Liverpool. He was the first child of healthy parents (the mother aged 19, the father aged 20), who were first cousins (Fig. 1). The mother's health was

good throughout the pregnancy, which was uneventful. The only radiograph taken was a chest film at 32 weeks' gestation, which was reported normal. There was no history of blood disease or any other serious illness in the family.

The infant was born normally at full term, weighed 6 lb. 14 oz. (3,120 g.), and appeared quite healthy. On the following day skin purpura and slight jaundice were noted, and examination revealed gross enlargement of the liver and spleen. Examination of blood showed haemoglobin 100%

FIG. 1.—Family tree: the arrow indicates the proband. All the individuals represented above are alive and well.

(14.8 g./100 ml); leucocytes 24,000/c.mm, with a normal differential count; platelets 165,000/c.mm. The child was transferred to Alder Hey Children's Hospital on the third day of life for investigation.

On admission there were petechiae and ecchymoses on the face and scattered petechiae on the trunk. No nodules were present in the skin, which was moderately icteric. No haemorrhages were seen in the mucous membranes. The liver was enlarged to 3 in. (7.5 cm.) and the spleen to 3½ in. (9 cm.) below the costal margins. No other abnormality was found and there were no signs of mongolism. The stools were of normal colour.

Investigations.—Haemoglobin 100% (14.8 g./100 ml.); reticulocytes 2%; the red cells showed anisocytosis and polychromasia; leucocytes 21,000/c.mm. (lymphocytes 53%, neutrophils 31%, eosinophils 8%, monocytes 8%); platelets fewer than 10,000/c.mm. Serum bilirubin 15 mg./100 ml. (conjugated 7 mg., unconjugated 8 mg.). Other liver-function tests were normal. Blood group B, Rh positive (mother O Rh negative without antibodies); direct Coombs test negative. Serological tests for syphilis and toxoplasmosis were negative in mother and child. No evidence of cytomegalic inclusion disease was found.

The infant's general condition remained about the same. No fresh petechiae appeared in the skin, but stools were occasionally blood-streaked. On the fifth day of life the haemoglobin was 86% (12.7 g./100 ml.) and the total leucocyte count was within normal limits, but blast cells (6%) were present. The white-cell count increased to 45,000/c.mm. on the eighth day, with 29% blast cells.

Marrow puncture was performed and, although no fragments were obtained, free cells were abundant. They were almost entirely of the lymphocytic series and over 75% had a primitive type of nucleus with an open chromatin network. Many contained one or two distinct nucleoli. The cytoplasm was scanty and limited to a very thin rim round the nucleus, and stained a pale blue. No megakaryocytes were present. The appearances were those of a blastic leukaemia, probably lymphogenous in type.

On the ninth day the leucocytes had increased to 140,000/c.mm. (blast cells 71%) and, after blood had been taken for chromosome studies, treatment was started with prednisolone, 50 mg. daily in divided doses. This was followed by a dramatic fall in the white-cell count to 26,000/c.mm. (blasts 51%) within 24 hours and to 2,500/c.mm. within 48 hours, with no blast cells present (see Fig. 2). The liver and spleen became reduced in size to 2 in. (5 cm.) below the costal margins. Fresh petechiae and ecchymoses appeared on the trunk and there was further gastro-intestinal bleeding. On the 12th day the haemoglobin was 25% (3.7 g./100 ml.) and a blood transfusion was given. The child's condition deteriorated and he died the following day.

Necropsy

The body was that of a small, wasted infant weighing 6 lb. 9½ oz. (2,990 g.). The skin was jaundiced, and numerous petechiae were present, especially over the lower abdomen. Stomach contents were present in the larynx, trachea, and main bronchi, but the lungs were fairly well aerated. The pleural cavities were dry. There was no abnormality of the heart or pericardium. Blood-stained fluid was present in the peritoneal cavity. Considerable haemorrhage had occurred into the lumen of the bowel and a large quantity of fresh clotted blood was present in the stomach and small intestines. There had also been bleeding into the walls of the stomach, pylorus, and ileum.

Apart from enlargement there was no obvious macroscopic abnormality of the liver and spleen, which weighed 235 g. and 45.5 g. respectively. There was no anatomical abnormality of the bile ducts, but the bile passages were filled with thick inspissated dark-green bile. The abdominal lymph nodes were enlarged, dark, and haemorrhagic. Death was attributed to haemorrhage into the gastro-intestinal tract.

The significant histological findings were confined to the abdominal viscera and bone-marrow. The pancreas was diffusely infiltrated by masses of mononuclear cells morphologically indistinguishable from blast cells. They mainly followed the line of the fibrous tissue septa, but had also invaded and destroyed the parenchyma in several places. They were uniform, and had a round or slightly reniform darkly staining nucleus and almost no cytoplasm. Occasional mitoses were present. Some of the pancreatic ducts were dilated.

The liver showed several features of an obstructive biliary cirrhosis. The parenchyma was divided into irregular lobules by bands of fibrous tissue spreading from the periportal areas. There had also been marked proliferation of bile-ducts, which were dilated and contained inspissated bile plugs. No bile retention had occurred in the bile canaliculi or in the liver cells. There was no inflammatory cell infiltration. A few foci of erythropoiesis were scattered throughout the sinuses.

In the spleen the architecture was normal, but there was much shrinking of the Malpighian bodies, which had lost a substantial proportion of their cell population. A few foci of erythropoiesis were present. Some focal haemorrhages had occurred in the sinuses. The marrow also showed depopulation of cells and focal haemorrhages. As in the spleen, there was no convincing evidence of active leukaemic infiltration.

In the kidneys there were, in addition to areas of erythropoiesis, small foci of large mononuclear cells similar to those in the pancreas, and they were usually associated with focal haemorrhages.

Chromosome Studies

Chromosome analysis was carried out from a sample of peripheral blood taken on January 31, 1961, and cultured, using a modified version of the technique of Moorhead *et al.* (1960). After differential centrifugation the concentration of leucocytes was 87,000/c.mm. and, as the time taken for cells to reach optimum rate of division is variable in different

leukaemic blood samples, cultures were set up as follows: (a) Diluted to 3,000 white blood cells per c.mm. with AB serum (70%) and T.C. 199 (30%) and incubated for a total period of seven hours, as for bone-marrow culture. (b) Diluted to 1,500 white blood cells per c.mm. with T.C. 199; one culture was incubated for two days and another for three days.

Very few dividing cells, none of which were countable, were found after the short-term culture, although this method has proved successful in other cases. More cells were seen in division in the two-day and three-day cultures,

Fig. 2.—Serial blood counts in relation to treatment.

but only 17 were countable; 15 of these were found in preparations made from the two-day culture. Seven cells were fully analysable and no evidence of any chromosomal abnormality could be found.

Samples of blood were also taken from both parents of the baby. These were cultured for the three-day period and gave good-quality preparations. From these only enough cells were counted and analysed to confirm a normal chromosome complement with the absence of any aberrations. Table I gives the actual chromosome counts made.

TABLE I.—Distribution of Chromosome Counts

	<45	45	46	47	Polyploid	Total	Analysed
Baby	2	1	12	1	1	17	7
Mother	1	1	30	2	0	34	5
Father	2	1	25	1	1	30	5

Discussion

Diagnosis of Congenital Leukaemia

The syndrome of jaundice, skin haemorrhages, and enlargement of the liver and/or spleen in the newborn is by no means uncommon and may present diagnostic difficulties. The initial clinical diagnosis in this case was cytomegalic inclusion disease. Some of the principal conditions which may present with this clinical picture are listed in Table II with some points of differential diagnosis. It will be seen that there are two

main groups—namely, infective conditions (viraemia, septicaemia, toxoplasmosis, syphilis, cytomegalic inclusion disease) and blood disorders (haemolytic disease, thrombocytopenic purpura, leukaemia).

In the present case the combination of anaemia, thrombocytopenia, and large numbers of immature white cells in the peripheral circulation, in conjunction with an active marrow, almost completely replaced by blasts, makes a diagnosis of leukaemia inescapable. It is, however, often demanded that there should be evidence of infiltration. In the present case the pancreas was extensively infiltrated—indeed, to such an extent that there must have been obstruction to a major bile-duct for long enough to cause biliary cirrhosis. It is

TABLE II.—*Differential Diagnosis of Congenital Leukaemia*

	Splenomegaly	Hepatomegaly	Purpura	Icterus	Leucocytosis	Anaemia	Thrombocytopenia	Other Diagnostic Features
Viraemia	+	+	+	+	+	+	+	Herpes — characteristic rash. Coxsackie—myocarditis. Virus studies
Septicaemia	+	+	+	+	+	+	+	Blood culture
Toxoplasmosis	+	+	+	+	±	±	±	Dye test and C.F.T. on mother and infant
Cytomegalic inclusion disease	+	+	+	+	+	+	+	Inclusion bodies in cells of urinary sediment. ? C.F.T.
Syphilis ..	+	+	—	+	+	+	—	Rash, snuffles, osteochondritis. Serology of mother and child. Bone x-ray film
Haemolytic disease	+	+	±	+	+	+	±	Rh incompatibility; Coombs test positive
Primary thrombocytopenia	±	—	+	—	—	±	+	Family history. Prolonged bleeding-time, capillary fragility, poor clot retraction, ? platelet agglutinins
Leukaemia	+	+	+	±	+	+	+	Skin lesions. Blast cells in blood and marrow

interesting to note that the spleen and marrow not only failed to show infiltration but even showed noticeable depopulation of cells. This was consistent with the remarkable fall in the peripheral white-cell count after steroid therapy.

Treatment of Congenital Leukaemia

Congenital leukaemia runs a very short course and there are few reports of attempts to treat it. Urethane (Taylor and Geppert, 1950) and A.C.T.H. (Lee and Ciner, 1957) have been tried without effect. In the present case high doses of prednisolone were followed immediately by a profound fall in the peripheral white-cell count, diminution in size of the liver and spleen, and disappearance of blast cells from the marrow. Death was attributable to gastro-intestinal haemorrhage, and post-mortem evidence of leukaemia was found only after careful search.

Aetiology of Congenital Leukaemia

Genetic and environmental factors have both been implicated in the genesis of leukaemia. The genetic element is seen in its purest form when there is a high familial incidence of leukaemia, as in the family described by Anderson (1951) in which five out of eight children were affected. In those less striking but more numerous sibships containing two or three affected individuals, the genetic factor is presumably strong. At the other extreme, leukaemia after high doses of ionizing radiation may be presumed to be of almost purely environmental origin.

In most instances, however, a combination of genetic predisposition and environmental trigger precipitates the clinical disease. In some individuals, notably mongols, the trigger fires at a light touch, their abnormal chromosomal constitution giving them a strong predisposition. Stewart (1961) has suggested the name *prezygotic* for those leukaemias which are predominantly of genetic causation, and *post-zygotic* for those attributable largely to prenatal or post-natal environment. By and large, prezygotic leukaemias are of lymphatic or stem-cell type and arise early in life, while post-zygotic cases are often myeloid and are seen principally in older children and adults. The discovery of the Philadelphia chromosome (Nowell and Hungerford, 1960; Baikié *et al.*, 1960) in chronic myeloid leukaemia is not inconsistent with this theory, since it is only the abnormal leucocytes and their precursors that contain the abnormal chromosome.

Because of its prenatal onset, congenital leukaemia might at first be thought likely to have a predominantly genetic basis, an idea strengthened in the present instance by the consanguinity of the parents. Although some cases of acute leukaemia have been reported with a variety of chromosomal aberrations in bone-marrow samples, others have shown no abnormalities (Ford *et al.*, 1958; Baikié *et al.*, 1959, 1961; Ford, 1960; Sandberg *et al.*, 1960). Unfortunately, marrow was not available for culture in the present case and it is not known whether the cytological condition of the marrow was truly reflected in the peripheral blood. From experience with both marrow and peripheral blood samples in other cases of acute leukaemia and from investigations already reported (Baikié *et al.*, 1961) it is evident that the findings in these two tissues may not correspond with one another.

Against a genetic basis for congenital leukaemia, parental consanguinity has not previously been reported, the disease has not been reported in an infant born to a leukaemic mother, nor has more than one case been reported in a sibship. Furthermore, a high proportion of authors have felt that their cases of congenital leukaemia were of myeloid type.

The causative factor or factors would therefore appear to operate between conception and birth. The most obvious possibility—ionizing radiation—has been excluded in this case, nor do bacterial or chemical toxins appear to have played any part.

It is of interest that leukaemia has only twice been described in stillborn babies (Koch, 1922; Bungeler, 1931). This observation, together with the fact that in this case the peripheral blood at birth was normal, suggests that there may be some restraining mechanism *in utero* which breaks down at birth. The remarkable effect of prednisolone on both peripheral blood and bone-marrow raises the possibility that maternal adrenal cortical hormones may play a part in this mechanism.

Summary

A case of congenital leukaemia is described in an infant whose parents were first cousins. Chromosome studies on peripheral blood revealed no abnormality in the child or either parent. The disease responded briefly but dramatically to treatment with prednisolone. The diagnosis, treatment, and aetiology of congenital leukaemia are discussed.

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Medical Memoranda

Fatal Case of Stevens-Johnson Syndrome after Sulphamethoxypyridazine Treatment

Sulphamethoxypyridazine, a long-acting sulphonamide, has been widely used since its introduction a few years ago. Increasingly frequent reports of reactions indicate that its use is not without risk, although few fatal reactions have been recorded. In the following case its administration was followed by an extensive bullous erythema multiforme (Stevens-Johnson syndrome) which ended fatally.

CASE REPORT

The patient, a 69-year-old woman, was admitted to hospital in August, 1959. Four weeks previously she had developed an influenza-like illness with pharyngitis and productive cough, for which she received oral penicillin V, 1 g. daily for three days, followed by sulphamethoxypyridazine, 0.5 g. daily for 19 days—that is, until the time of admission.

Three days before admission she awoke with an intensely irritating bilateral conjunctivitis, which was later associated with a purulent exudate and mild photophobia. An erythematous skin eruption developed, involving most of the body. This progressed, and in places the epidermis stripped off, exposing raw bleeding areas. The buccal mucosa was extensively ulcerated. The urethra was also involved, causing severe dysuria, and eventually urinary retention with overflow.

There was no personal or family history of allergy or previous drug sensitivity. Over the previous five years the patient had received four short courses of treatment with "sulphatriad" and sulphathiazole for throat and gastrointestinal infections.

In 1945 a hysterectomy had been performed for carcinoma of the body of the uterus, which was followed by a course of deep x-ray therapy.

On examination the patient, a moderately obese elderly woman, had a temperature of 101° F. (38.3° C.). There was a widespread bullous skin eruption involving most of the body, with excoriation around the mouth and eyelids. Stripping of the epidermis occurred wherever sliding pressure was applied to affected areas of skin (Nikolsky's sign). The conjunctivae were injected, with purulent exudate, and there was extensive corneal ulceration. The tongue and buccal mucosa were grossly ulcerated, and swallowing and speech soon became very difficult. The vulva was intensely inflamed and ulcerated. There were no other significant findings

apart from a midline lower abdominal surgical scar with some surrounding atrophy and fibrosis from previous deep x-ray therapy.

Investigations.—In the Stevens-Johnson syndrome the laboratory findings do not reflect the severity of the constitutional disturbance. In this case investigations for the most part gave normal results: haemoglobin, 14.9 g./100 ml.; leucocyte count, 5,200/c.mm. (band neutrophils 30%, segmented neutrophils 44%, lymphocytes 22%, monocytes 4%); E.S.R. 11 mm. in 1 hour (Westergren); total serum proteins, 6.4 g./100 ml. (albumin 3 g., globulin 3.4 g.). Blood urea initially was 63 mg./100 ml. The serum electrolytes were normal. The urine contained a trace of protein and showed red cells in the deposit. No pathogens were isolated on bacteriological culture of swabs taken from throat, conjunctivae, skin vesicles, and vulva. A chest x-ray film showed a rounded opacity 2 cm. in diameter in the left upper zone, but was otherwise clear.

Treatment was started with erythromycin 2 g. daily and prednisone 40 mg. daily, which was later increased to 80 mg. daily. On the higher dose of prednisone the temperature returned to normal. As the patient could take only small amounts of fluid by mouth, fluids were given intravenously to maintain an adequate fluid balance. In spite of treatment her condition steadily deteriorated. A toxic auricular fibrillation developed which was controlled with digoxin. She died 12 days after admission.

Necropsy Findings.—The skin ulceration involved 40% of the body surface. There were also ulcerated areas in the mouth, conjunctiva, cornea, vulva, vagina, and urethra. Histologically the picture was that of an acute polymorphonuclear inflammatory response with ulceration. The rounded opacity present on the chest x-ray film was found to be due to a tumour deposit, histologically consistent with metastasis from endometrial adenocarcinoma. A similar tumour deposit was found in the vault of the vagina. The uterus and cervix were absent. The heart was normal, the trachea and bronchi were normal, and there was no evidence of pneumonia, although the lungs showed some congestion. There was a small acute erosion in the stomach just above the pylorus.

COMMENT

The association of bullous erythema multiforme with extensive ulceration of mucous membranes constitutes the Stevens-Johnson syndrome. The classification and terminology of this condition is rather confused. The Stevens-Johnson syndrome, ectodermosis pluriorificialis, Behcet's syndrome, and possibly Reiter's disease may all be variants of erythema multiforme, and it has been suggested that the term "muco-cutaneous ocular syndrome" could be used to include all these disorders (Robinson and McCrumb, 1950).

The aetiology of the Stevens-Johnson syndrome is still obscure, but often it seems to occur as a hypersensitivity reaction to drugs, particularly sulphonamides and phenobarbitone (Mauriello, 1954). It is hard to escape the conclusion that sulphamethoxypyridazine was responsible for the skin eruption in this patient. Nine previous cases of severe bullous erythema have been reported in association with this drug (Lindsay *et al.*, 1958; Salvaggio and Gonzalez, 1959; Garner, 1959; Yaffee, 1959; Maher-Loughnan and Tullis, 1960). One of these proved fatal, this case being complicated by a septicaemia.

It is becoming increasingly evident that sulphamethoxypyridazine may cause untoward effects, in general similar to those caused by earlier sulphonamides. Minor effects that have been reported are headache, nausea, anorexia, fever, giddiness, joint pains, and mild skin eruptions. Their incidence is difficult to assess: estimates vary from 3.6% (Stewart *et al.*, 1960) to 15%