ORIGINAL ARTICLES

The Cornelia de Lange Syndrome:

Clinical and Cytogenetic Interpretations

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ABSTRACT

A euploid/aberrant double stem line mosaicism was found in two cases of the de Lange syndrome with severe abnormalities. In both cases the structural heterozygosity of the aberrant stem line involved, apparently, the loss of chromosomal material from a smaller autosome of Group (6-12) X, probably No. 11. Differences in the cultural characteristics of de Lange cells suggest that the aberrant stem line may not proliferate in culture, so that mosaicism may not be detected. Moreover, the mosaicism may not be present in all tissues, resulting in normal cytogenetic findings as noted in a third case studied. Our findings suggest that the de Lange syndrome is the phenotypic expression of chromosomal mosaicism.

SOMMAIRE

Dans deux cas de syndrome de Lange comportant des anomalies graves, on a observé de la mosaïque à deux lignées aberrantes euploïdes. Dans les deux cas, l'hétérozygosité structurale de la lignée aberrante impliquait, apparemment, une anomalie consistant dans la perte de matériel chromosomique d'un autosome plus petit du groupe 6-12, probablement le No 11. Les différences dans les caractéristiques des cultures des cellules de Lange permettent de croire que les lignées aberrantes ne poussent pas en culture, de sorte que la mosaïque ne peut pas être décelée. Par ailleurs, la mosaïque ne peut pas être présente dans tous les tissus, ce qui se traduit par des constatations cytogénétiques normales, comme on l'a observé dans un troisième cas. Nos observations laissent présumer que le syndrome de de Lange est l'expression phénotype d'une mosaïque chromosomique.

DE LANGE[1]† has generally been credited with the first description of a syndrome encompassing severe mental and growth retardation, peculiar facies, and anomalies of the extremities which she named Typus Degenerativus Amstelodamensis. All known cases previous to 1963 were recorded in European literature but over 20 cases have been reported or identified by American investigators since 1963.

The strikingly similar facies of the patients results from the constant finding of brachymicrocephaly, confluent eyebrows, long eyelashes, antimongoloid slant of the eyes, depressed nasal bridge, anteverted nostrils, thin broad lips with downturned angles and micrognathia. Micromelia, frequently associated with oligodactylia or phocomelia of the upper extremities and syndactylia of the second and third toes, is another consistent finding in the spectrum of anomalies. The above findings, when noted in an infant with a low-pitched cry, are frequently sufficient to confirm the diagnosis. The presence of widespread defects involving derivatives of all three germ layers and the rarity of the syndrome suggest the possibility of a chromosomal abnormality, but the majority of workers have reported normal findings.[2-10] Jervis and Stimson[11] and Massimo and Vianello[12] reported a variable percentage of cells contained an acentromeric fragment and Geudeke, Bijlsma and de Bruijne[13] reported B/G translocation in one of three cases. Although subtle chromosomal aberrations are not ruled out by cytogenetic studies to this date, Opitz *et al.*[9] believe that the frequency and the occasional familial incidence of this syndrome are compatible with autosomal recessive inheritance.

The purpose of this paper is to report three cases, of which two are considered to be typical examples of the Cornelia de Lange syndrome and the third is probably a *forme fruste* of this syndrome. In addition, evidence will be presented indicating that the typical Cornelia de Lange syndrome is the phenotypic expression of chromosomal mosaicism. No effort will be made to elaborate on the biochemical and pathological findings

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of this syndrome since they have been fully described elsewhere.[1, 4, 7, 8, 10, 11]

METHODS

A micromethod* was employed in all three cases. Between 0.15 and 0.20 ml. of heparinized whole blood was added to a completed medium consisting of 5 ml. of Eagle's minimum essential medium, 0.5 ml. of bovine serum, 80 μg. of neomycin sulfate and 0.1 ml. of Bacto phytohemagglutinin M (Difco). The cells were incubated for a period of 48 to 72 hours, following which 0.05 ml. of 0.004% colchicine solution was added per 5 ml. of culture medium. Incubation was continued for another four hours, and the cells were harvested according to previously described methods.

In Case 1 only two of the four cultures yielded sufficient mitoses for analysis. This relatively poor growth rate was duplicated in cultures from Case 2 and was originally attributed to technical error or the inability to obtain a suitable cell inoculum. However, later studies indicate that the growth requirements of leukocytes derived from these patients are different from those of normal cells and the poor growth response, under the conditions of this study, are believed to be a reflection of inherent metabolic errors. The same problems were not encountered in cultures obtained from Case 3 and one culture was deemed sufficient for analysis.

CLINICAL FINDINGS

CASE 1.—The propositus (Fig. 1), born May 25, 1963, was admitted to Fairview State Hospital on August 28, 1964, and is the fourth child of phenotypically normal parents. The mother and father were 27 and 32 years of age, respectively, at the time of the patient's birth. The pregnancy was full term and the mother stated that she had severe nausea and vomiting during the first trimester and a persistent "cold" during the last five months of pregnancy. Delivery was uneventful and birth weight was recorded as 3 lb. 2 oz. The mother was unable to give an accurate postnatal history, but the propositus remained in the hospital for a longer period than normal and was discharged with a weight of 5 lb. 2 oz. He continued to thrive poorly, had frequent episodes of vomiting and cyanosis due to aspiration of gastric contents and was noted to be "poorly nourished" at the time of examination in the Preadmission Service. At this time, the mother volunteered the information that she had been given an unknown medication by her physician to bring on her menstrual periods during the first trimester of the pregnancy. Exposure to irradiation was denied.

The paternal grandparents were first cousins and a paternal uncle was transferred to Fairview State Hospital (June 4, 1959) from another state hospital with a diagnosis of familial mental retardation (I.Q. 28). Another paternal uncle is "slow to learn" and a paternal aunt is evidently retarded, but neither is hospitalized. One other paternal uncle is phenotypically normal. There is no history to suggest that retarded members on paternal side are afflicted with the Cornelia de Lange syndrome. The three siblings are normal.

The patient was 24½ months of age at time of examination and weighed 10 lb. 3 oz. He was obviously severely retarded (I.Q. 10). Head circumference was 15 inches with a brachymicrocephalic configuration. Flattening of the occiput was quite remarkable. The scalp hair was long, fine, and sparse, and extended on to the forehead and posteriorly more than normal. Venous dilatation of the frontal and temple areas was quite marked, as was facial cyanosis. An antimongoloid slant of the eyes was quite evident. The eyebrows were bushy and confluent, and the eyelashes long and delicate. The nasal bridge was depressed, nostrils anteverted and flared, giving a "beak-like" appearance. Micrognathia, of a moderate degree, was also noted. The palate was high, the tongue small and

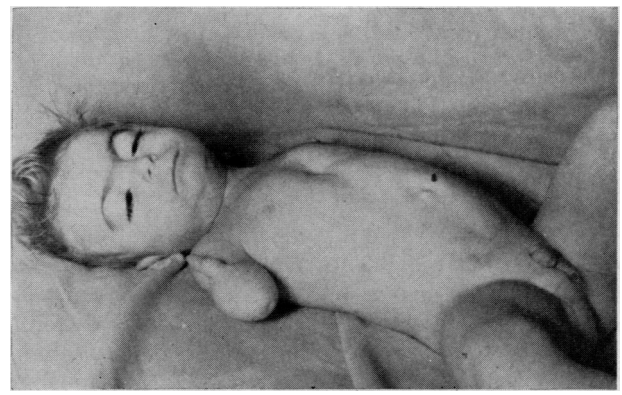

Fig. 1.—Case 1.

nonfissured. The lips were thin and down-turned at the angles, and hard paramedian spurs were quite remarkable at the inferior margin of the symphysis mentis. A single lower central incisor had partially erupted. Ptosis of both upper eyelids and bilateral optic atrophy were noted. The chest circumference was 15 inches and the biacromial diameter was 7 inches. A minimal pectus excavatum was evident. The areolae were small and widely spaced. No cardiac or pulmonary abnormalities were detected. Abdominal circumference was 13 inches. No intra-abdominal masses were detected; the liver, kidney and spleen were within normal limits. Bilateral undescended testes and a small hemangioma (2 mm. in diameter) at the median raphe of the scrotum were noted.

Bilateral phocomelia of the upper extremities was present. Neither elbow could be extended owing to skin webbing, and the right forearm was extremely short and terminated in a single digit.

*We are indebted to Dr. A. Teplitz, City of Hope Medical Center, Duarte, California, for this modification of the method of Arakaki and Sparkes (*Cytogenetics* [Basel], 2: 57, 1963).

The left forearm was approximately twice the length of the right forearm and terminated in two vestigial digits. Micromelia was pronounced in the lower extremities and there was partial syndactylia of the second and third toes. The skin on the plantar surfaces was quite smooth and dermal ridging was barely detectable. Bilateral equinovarus deformities and hypoplasia of the tibiae and fibulae were easily discernible.

The skin generally was smooth, lacked substance and cutis marmorata was generalized. Hirsutism of the trunk was not noted and the patient's cry could be best described as a low-pitched whimper. Generalized hypotonicity was also evident. He responded to auditory stimuli.

A series of skull radiographs (August 28, 1964) revealed that the posterior fontanelle was closed. All major sutures remained open, as did the anterior fontanelle. No other abnormalities were noted. There was congenital absence of one of the long bones in the lower leg, bilaterally. It was difficult to state with any degree of certainty whether the single bone represented tibia or fibula. Forearm development was limited to one major long bone, which was hypoplastic. There was marked hypoplasia and aplasia of the carpal, metacarpal and phalanges, bilaterally. Development of the hand and wrist on the right was limited to a single phalanx and was presumably the residuum of the thumb. Significant bone or joint abnormality was not identified on an anteroposterior radiograph of the pelvis. Bilateral equinovarus deformities were noted. The number of small bones was normal. The chest radiograph showed no remarkable findings. No abnormalities were demonstrated on a gastrointestinal radiologic series.

The patient has had a low-grade microcytic anemia of 10 to 11 g. % since admission to the hospital. Routine urinalyses have been within normal limits. Routine chemistries have been within normal limits, but additional biochemical studies are being completed and will be reported at a later date. Buccal smears showed less than 4% chromatin bodies.

The patient has continued to thrive poorly and has had repeated respiratory infections since admission to hospital. He is unable to sit and has otherwise developed poorly and requires constant nursing care.

CASE 2.—The propositus (Figs. 2 and 3) was born on November 24, 1957. Pregnancy was uncomplicated except for bleeding in the first trimester. He is the third of four children, the other three being normal. There is an interval of approximately seven years between the second-born and the propositus. A spontaneous uncomplicated abortion preceded his birth by approximately one year and this was followed by a curettage approximately two months later. The mother was 29 years of age and the father 32 years of age at the time of his birth. Exposure to irradiation was denied. Birth weight was recorded as 3 lb. 11 oz.

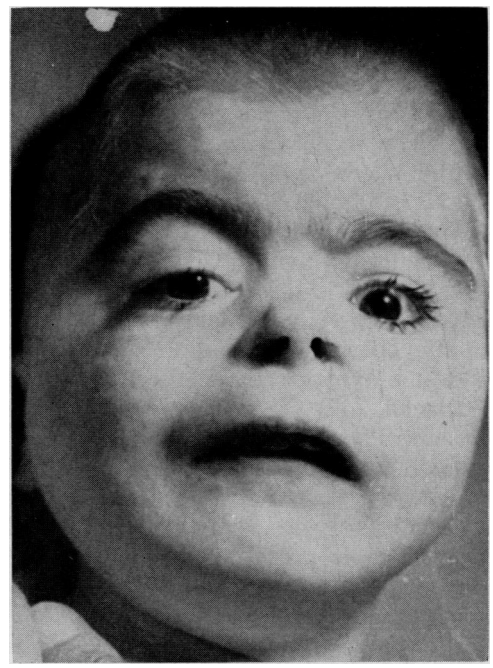

Fig. 2.—Case 2. Facial features are quite similar to those of Case 1.

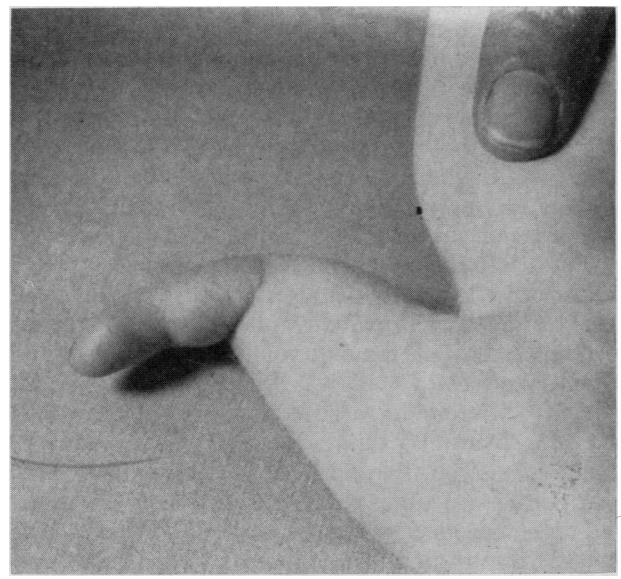

Fig. 3.—Phocomelia and oligodactylia as seen in Case 2.

There is no history of retardation or congenital abnormalities on either side of the family. Consanguinity was denied.

The patient was examined on March 25, 1965, at which time his weight was 26½ lb. and his length 36 inches. He was severely retarded (I.Q. 22) and sat alone unaided. His movements were fairly well co-ordinated and he was able to grasp small objects with his left hand. His cry was low-pitched, and no diminution of auditory acuity was noted. His head measured 17 inches in circumference and was brachymicrocephalic in configuration. The occiput was flattened on the left and the hair

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was rather coarse and extended lower on the forehead than normally. The pinnae were small and low-set. The eyes had a typical antimongoloid slant, and there was ptosis of the upper eyelid on the right. The right malar arch and orbit were hypoplastic. There was diminution of ocular movements with probable paresis of the left lateral rectus. The pupils were eccentrically placed and dilated irregularly. Lens opacities were noted on the right. The optic discs appeared to be normal, and there was no venous dilatation. The nasal configuration, eyebrows, and eyelashes were typical of this syndrome. Teeth were definitely hypoplastic, widely spaced, and the gingival margins thicker than normal. The palate was somewhat high, the tongue small and non-fissured. Micrognathia of a moderate degree was noted, but mandibular spurs were absent, as were venous dilatation of the frontal and temple areas and facial cyanosis. The chest measured 21 inches in circumference and the biacromial width was 9 inches. The areolae were hypoplastic and widely spaced. No cardiac or pulmonary abnormalities were noted. The abdomen was 19 inches in circumference. The umbilicus was remarkably small. No intra-abdominal masses could be detected, and liver, kidney and spleen were normal. A herniorrhaphy scar was noted on the right; the testes were undescended.

Phocomelia of the right upper extremity was extreme, with the right forearm terminating in a single digit. The right elbow could not be extended owing to cutaneous webbing. Micromelia was pronounced on the left and a simian line was present. Clinodactyly of the fifth digit was easily discernible and the distal phalanx was vestigial. The thumb was proximally placed and the thenar eminence smaller than normal. Syndactylia of the second and third toes was barely discernible but present. Micromelia of the lower extremities was marked. Dermal ridging on the plantar surfaces was less marked than normal, and generalized cutis marmorata was quite striking. Little moisture could be detected, and the substance of the skin was grossly diminished. Hypoplasia of the tibiae and fibulae was less remarkable than in Case 1 but evident. A large amount of fine lanugo was noted on the posterior trunk.

The roentgen findings were similar to those in Case 1.

The patient has continued to have marginal anemia (approximately 10 to 11 g. %) since admission to hospital. Routine urinalyses have been within normal limits. Routine chemistries have been within normal limits, except for reversal of the albumin/globulin ratio. Buccal smears showed less than 5% chromatin bodies.

The patient has required continuous nursing care since admission to the hospital and has had numerous respiratory infections. He is able to sit by himself and move about in a playpen but he is non-ambulatory. There has been no change in his grossly retarded growth pattern.

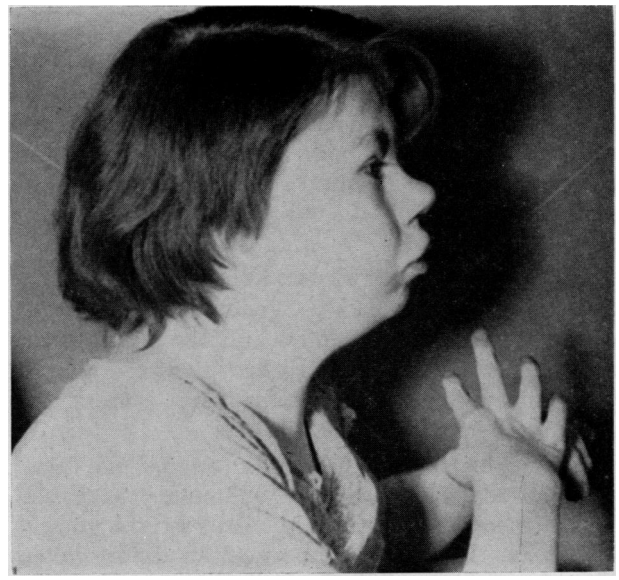

Fig. 4.—Profile of Case 3.

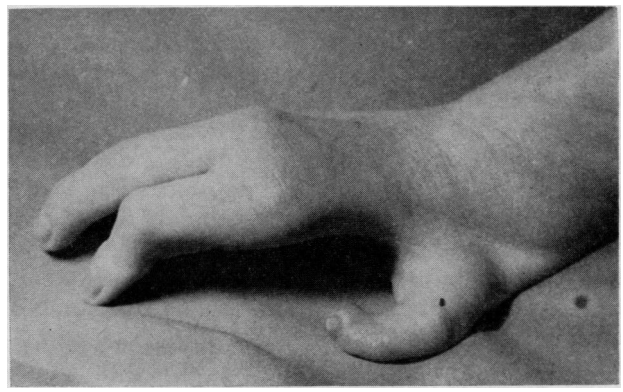

Fig. 5.—Left hand of Case 3 demonstrating aplasia of the fourth metacarpal and digit. Clinodactyly of the fifth digit is clearly evident.

CASE 3.—The propositus (Figs. 4 and 5) was admitted to Fairview State Hospital on May 24, 1963. She required total nursing care and was non-ambulatory. She was born on April 7, 1949, and delivery was approximately two weeks premature. The pregnancy was uncomplicated and delivery was spontaneous. There was no exposure to irradiation preceding or during pregnancy. The mother was 26 years of age and the father 32 years of age at the time of birth of the propositus.

The propositus has one sister, 20 years of age, who has been treated for "an underactive thyroid" for 10 years. The father died at the age of 42 as the result of "high blood pressure" but no autopsy was performed. The mother stated that many members on the paternal side were quite short but there was no history of abnormalities. Consanguinity was denied.

At time of examination, March 29, 1965, the patient weighed 76 lb. and was 54 inches tall. Head circumference was 19 inches and was roughly of microdolichocephalic configuration. The hair was coarse and extended frontally to more than the normal degree. The size and shape of the ears were

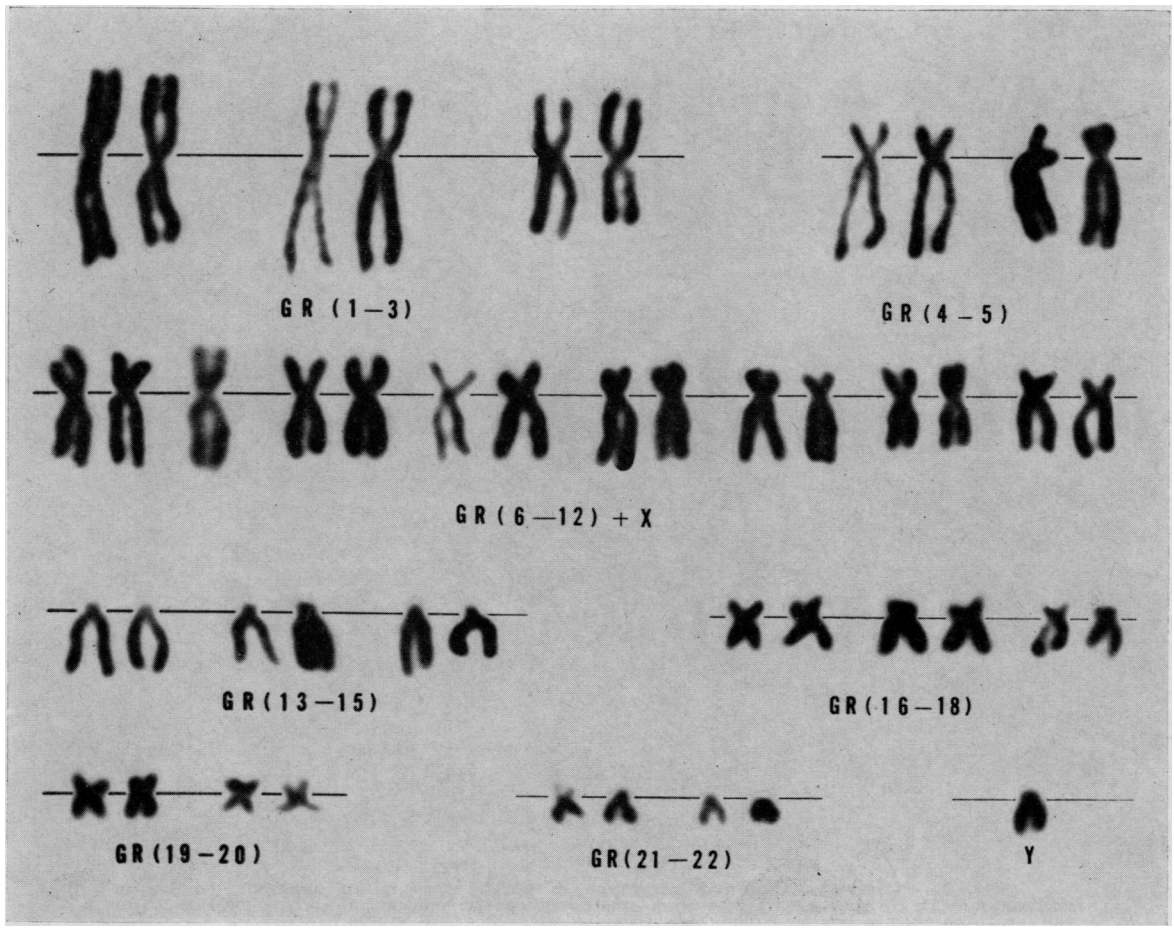

Fig. 6.—(Case 1). Normal karyotype. (Original magnification, × 5800.)

normal but the left auditory canal was atretic. A slight divergent strabismus was noted. Funduscopic examination revealed nothing except a mild degree of myopia. The face was asymmetrical in that the left malar arch and maxillary area were hypoplastic. The lips were thicker than one would have expected in the Cornelia de Lange syndrome, and down-turning at the angles was not evident. A mild degree of micrognathia was present. Bilateral paramedian spurs at the inferior margins of the symphysis mentis were easily palpated. The teeth were irregular but not hypoplastic and the palate was high. The nose did not have the typical configuration of the Cornelia de Lange syndrome, in that the alae nasi were much larger than would be expected. The nostrils, however, were anteverted and the nasal bridge depressed and there was incomplete fusion of the nasal bones along their whole length. The eyebrows were heavy and confluent in the midline, the eyelashes were long, and the eyes had a slight antimongoloid slant. Chest circumference was 32½ inches and the biacromial diameter was 12 inches. Slight breast development was noted on the right, but the left areola was hypoplastic. No abnormal cardiac or pulmonary findings were noted. The abdomen was 26 inches in circumference and the umbilicus was quite small. No abnormal findings were detected on abdominal examination.

Micromelia was quite remarkable in both upper extremities. The fourth digit and metacarpal were missing on the left hand and clinodactyly of the fifth digit was clearly evident. The latter digit was quite cyanotic. On the right the terminal phalanges of the fifth digit were hypoplastic and a simian line was present. The thumbs were proximally placed. Mild flexion contractures of the elbow were present bilaterally. The lower extremities demonstrated typical findings of the de Lange syndrome in that there was a partial syndactylia of the second and third toes, and micromelia was marked. The fourth digit, bilaterally, was hypoplastic and upturned. Flexion contractures of both knees were evident.

The skin was smooth and dry, and lanugo was present on the lumbar area. Beginning development of axillary and pubic hair was noted.

Clinical impressions were confirmed by radiographic studies. A gastrointestinal series was within normal limits.

The hemoglobin value remained at 10 to 11 g. % and urinalyses have been reported as normal. Buccal smears showed 36% chromatin bodies.

At birth, the propositus weighed 3 lb. 14 oz., and respiratory difficulty was experienced during the neonatal period. She was released to the mother's care after five weeks' hospitalization, at which time she weighed 5 lb. She had three episodes of

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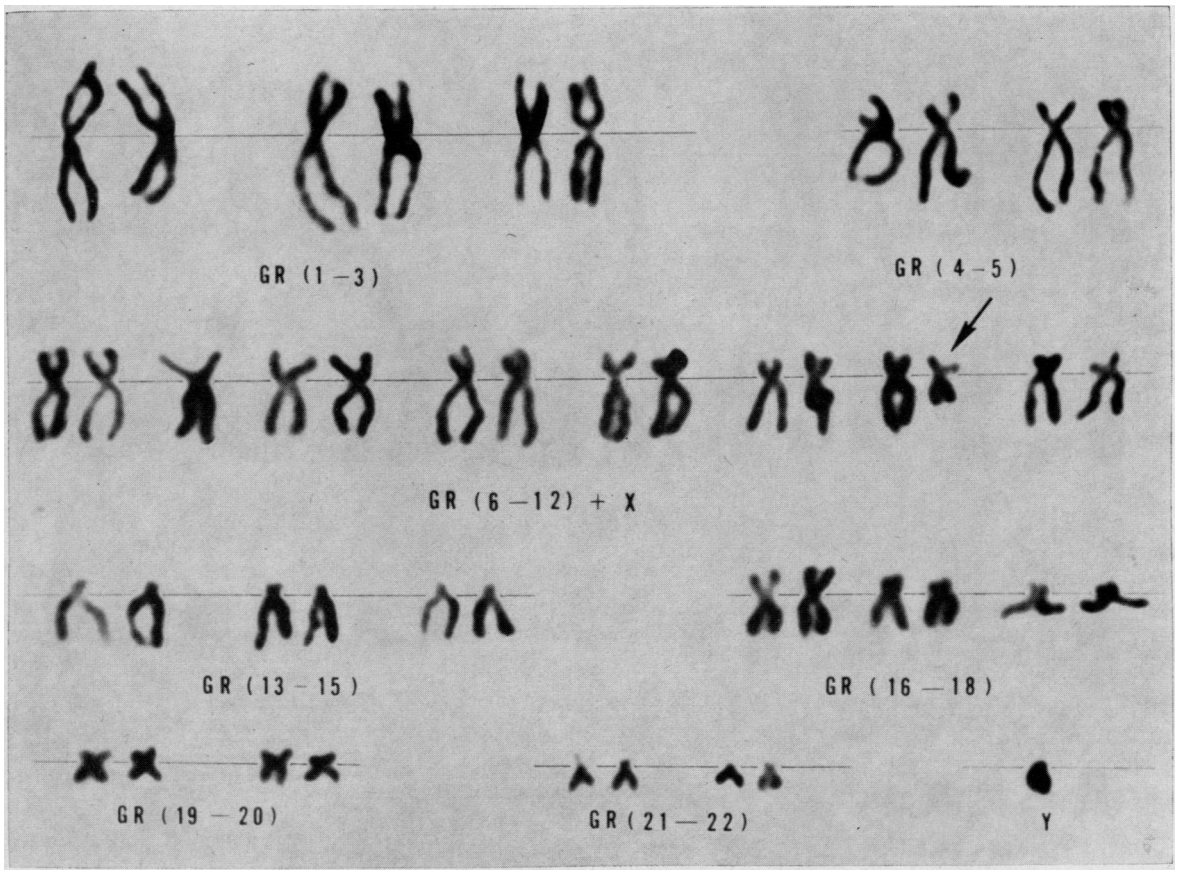

Fig. 7.—(Case 1). Abnormal karyotype in which there is an apparent deletion of chromosome 11 (arrow) giving rise to a small metacentric chromosome. This appears to be the most appropriate grouping (see text). (Original magnification, × 4600.)

pneumonia during the first year of life and vomiting of undetermined origin was frequent during the first two years of life. The patient was kept at home until 13 years of age, was not toilet-trained, and never made any attempts at walking.

The patient's menarche occurred approximately six months ago and she has had irregular menses (four to six weeks) since then. She continues to require total nursing care and communicates only by low-pitched grunts. I.Q. is estimated at 4.

In summary, the patient's growth has not been retarded to the degree noted in the first two cases. The facial complex of abnormalities is incomplete, no significant ocular abnormalities were noted and limb abnormalities are much less marked. Functioning ovarian tissue is confirmed by the patient's menstrual periods.

CYTOGENETIC FINDINGS

At least 40 cells were counted in each case and the modal number of chromosomes was 46 in all three cases. This was confirmed by examination of 25 enlarged prints of metaphase plates in each series.

CASE 1.—Seventeen karyotypes were examined, of which 14 were apparently within normal limits. In three karyotypes, Gr(6-12)X could not be matched satisfactorily. One chromosome of this group, probably chromosome 11, is abnormal in that a segment of the long arms has been deleted or translocated to another chromosome. This has resulted in a small metacentric chromosome approximately the size and morphology of Gr(16-18). This is apparently a deletion, since none of the other chromosomes are abnormal with respect to size and morphology. In Figs. 6 and 7 the normal and abnormal karyotypes are compared. The possibility was considered that the chromosomes assigned to position 16 could be more appropriately placed in position 11. Examination of the two other abnormal karyotypes showed that the two smaller metacentric chromosomes definitely belong to Gr(16-18), confirming the grouping as depicted. No acentromeric fragments were noted in any of the metaphase plates.

CASE 2.—Nine karyotypes were examined, of which three were within normal limits. Euploid and aberrant karyotypes were identified in each of two cultures. In six karyotypes, one member of Gr(6-12)X was missing, and an additional fragment, approximately the size of the short arms of chromosome 11, was noted. In addition, only three members of Gr(4-5) could be identified. A large chromosome, with long arms identical in length to that of the largest member of Gr(4-5), was found, but the short arms were much longer than normal.

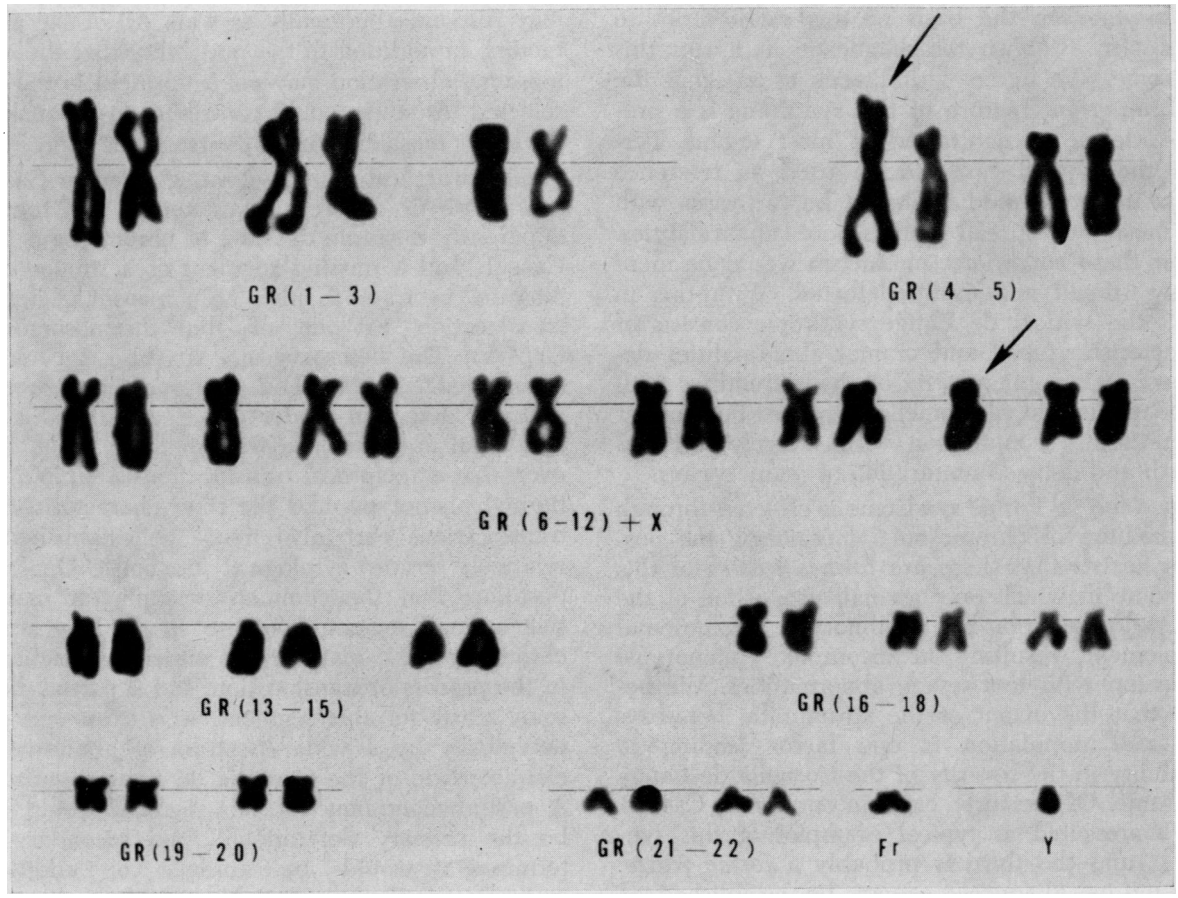

Fig. 8.—(Case 2). Abnormal karyotype in which there is an apparent translocation to one member of Gr(4-5) and a single No. 11 chromosome (upper and lower arrows, respectively). A fragment, possibly representing the short arms of the missing chromosome 11, is seen at Fr. See text for interpretation. (Original magnification, × 5800.)

This chromosome apparently belongs to Gr(4-5) and is a recipient of a translocated segment from an unknown donor, possibly the long arms of chromosome 11. In one metaphase plate, condensation of the translocated segment was much more marked than in the long arms and its margins could be clearly delineated. The fragment is slightly larger than Gr(21-22) chromosomes, and a centromere is probably present, since a well-defined constriction can be identified. The most appropriate grouping is as depicted in Fig. 8. The structural heterozygosis has most likely resulted from a reciprocal translocation between one member of Gr(4-5) and chromosome 11. Other interpretations, of course, are possible but would appear to be less likely. A well-defined constriction is noted in the short arms of abnormal member of Gr(4-5) in Fig. 8. No abnormalities were noted in any of the other groups.

CASE 3.—No abnormalities were detected in 13 karyotypes examined.

DISCUSSION

The importance of strict adherence to the criteria for diagnosis of the Cornelia de Lange syndrome became evident early in the course of this investigation. The three cases reported herein were selected from four cases presented at one of our consultant's clinic as examples of the Cornelia de Lange syndrome. The fourth case, that of a 29-year-old female with some of the facial features and limb abnormalities seen in this syndrome, was excluded from the series on the basis of incomplete phenotype, the lack of a clinical course characterized by severely retarded growth and repeated infections, and the demonstration of chromosomal abnormalities compatible with previously described aneuploidies. This experience caused us to be much more guarded with respect to the diagnosis of this syndrome, and to conclude that this diagnosis must be questioned when the abnormalities are not severe and involve many derivatives of all three germ layers. Noe[8] noted that only two, not including one of the four cases reported by Geudeka, Bijlsma and de Bruijne,[13] of more than 20 cases reported in European literature by the end of 1963 had phocomelia and oligodactylia, whereas approximately half of the cases reported by American workers have had severe limb abnormalities. Many factors may lead to this discrepancy, but from the standpoint of detecting diagnostic biochemical aberrations, which must exist in this syndrome, and confirmation of cytogenetic findings, the importance of selecting homogeneous samples for study cannot be understated.

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We hesitate on the basis of this small series to attempt to establish the diagnostic limits for this syndrome. We agree with Ptacek *et al.*[7] that the most important feature of this syndrome is a profound delay in maturation of most organs. Perhaps the typical cases to be used as reference points in future studies should be the cases with the most widespread and severe abnormalities. Under these conditions, mosaicism would be more widely spread and easily detected. With this in mind, the typical de Lange syndrome consists of characteristic facial and cranial abnormalities described earlier, micromelia of the extremities with oligodactylia and phocomelia of one or both upper extremities in conjunction with severely retarded growth and delayed maturation of many organs.

Since the de Lange syndrome is effected through the medium of chromosomal mosaicism, the possibility exists that there are *formes frustes* of this syndrome in which only a small proportion of the total cell population has an abnormal chromosomal complement, resulting in incomplete phenotypic expression with less severe abnormalities. We believe that the extent of the structurally heterozygous cell population is one factor leading to variability in the severity of the Cornelia de Lange syndrome. Of the three cases in our series, Cases 1 and 2 are cited as typical examples of this syndrome, and the third is probably a *forme fruste.* A definitive diagnosis cannot be established in Case 3 until we are able to demonstrate chromosomal mosaicism.

Previous reports[2-10] of apparently normal chromosomal findings in the Cornelia de Lange syndrome are not irreconcilable with our findings.* There have been few reports on the responsiveness of human cells with chromosomal aberrations to mitogenic stimuli and differing cultural conditions. The possibility exists that the proliferation of aberrant cells may lag considerably behind that of normal cells in short-term cultures, so that their percentage at the time of examination may be considerably reduced. In this investigation, leukocytes derived from Cases 1 and 2 certainly did not proliferate as rapidly as most cells and in a number of cultures little growth occurred. Technical errors were ruled out and continuing investigations are suggestive that the leukocytes (probably the aberrant stem lines) derived from these patients are quite sensitive to changes in media constitution. If the percentage of aberrant cells progressively decreases, the chance of detecting mosaicism would be remote if only a few karyotypes are examined as has been reported in two series.[5, 6] Other workers have noted that aneuploid cells, which differ from the normal in chromosomal number only, respond differently to mitogenic stimuli,[14] and cells that carry unbalanced translocations or deletions may respond abnormally as well. All of the above factors, in addition to the possibility that the chromosomal aberration may not be present in the cells selected for study, could contribute to the inability to detect mosaicism in patients of this type.

The structural heterozygosities noted in Cases 1 and 2 are of different character in that there is apparently a simple deletion of chromosome 11 in Case 1, and a missing segment of a similar chromosome in Case 2, plus an apparent reciprocal translocation to one of the chromosomes of Gr(4-5). The heterozygosity in Case 2 could be interpreted as a euploid reciprocal translocation and its detection under these conditions a coincidental and unrelated finding. The proof, however, that a reciprocal translocation is euploid, is a normal phenotype, and the finer characteristics of translocations certainly cannot be established by relatively crude cytological methods. One may postulate that the common denominator may be that a small segment, possibly in the long arm of chromosome 11, may be lost either by deletion or in the process of translocation, and if partial monosomy exists for this segment, it is expressed phenotypically by a wide spectrum of abnormalities characteristic of the Cornelia de Lange syndrome. A partial monosomy for this segment would then be the primary determinant, and secondary determinants would be absence of additional juxtapositioned segments. If additional segments were lost from other chromosomes, e.g. Gr(4-5) during the process of translocation, the picture would be increasingly complicated. Since the loss of chromosomal substance has a near lethal effect as a mosaic, one may postulate further that the presence of this aberration in all cell populations would be lethal, and the ameliorating effect of a normal cell population permits survival of the organism. The latter postulation is not without precedence, since the presence of a normal cell population has been shown to have a similar effect upon the phenotypic expression of trisomy 21[15, 16] and Patau[20] has come to a similar conclusion with respect to other types of mosaicism.

In the three cases studied cytogenetically by Geudeke, Bijlsma and de Bruijne,[13] a definite structural heterozygosity was noted in one. This may result from a reciprocal translocation between Gr(21-22) and Gr(4-5), and Opitz *et al.*[9] suggests that this may be a coincidental and unrelated finding. This assumption cannot be made unless similar translocations can be detected in the phenotypically normal parents or sibs. The interpretation offered by Geudeke, Bijlsma and de Bruijne[13] of the published abnormal karyotype appears to be the most apt, but other interpretations and groupings are possible. The findings of Jervis and Stimson[11] and Massimo and Vianello[12] are more difficult to correlate with ours in that an additional chromosomal fragment was noted in some of the cells examined.

*An additional case with a normal chromosome complement is reported briefly by Dumars, K. W. and Gaskill, C.: *The Human Chromosome Newsletter,* 12: 2, 1964.

However, their reports do indicate difficulty in chromosomal grouping and possible mosaicism.

Etiology.—The etiology of chromosomal mosaicism in the human is poorly understood, but reports within recent years indicate mosaicism may result from multiple fertilization of the products of oogenesis. Zuelzer, Beattie and Reisman[17] describe a type of generalized mosaicism that could only have resulted from such a sequence of events, possibly fertilization of an unextruded second polar body. Their interpretations are given support by the reports of Austin and Amoroso[18] and Ohno, Klinger and Atkin[19] who noted that the second polar body is extruded after fertilization and that fertilized ova may be associated with as many as three polar bodies. Since the polar bodies are grossly deficient in nutrients, the possibility that a normal rate of cell division could follow fertilization of these normally discarded bodies would appear to be remote, and consequently their contribution to the total cell population would be small. However, the importance of intracellular storage of nutrients in the oocytes may be much less in the higher eutherian animals than lower forms and the frequency of multiple fertilization of the products of oogenesis, leading to mosaicism, may be much higher than previously imagined. In order that mosaics with normal and aberrant stem lines may result as in Cases 1 and 2, either the ovum and a polar body or the fertilizing spermatozoa must carry dissimilar chromosomal complements, one of which must be euploid and the other unbalanced by reason of a deletion or a reciprocal translocation involving chromosome 11. However, the true incidence of this anomalous type of fertilization is unknown since only two verified cases have been reported[17] and it would be unwarranted to assume that mosaicism in the de Lange syndrome has its origin in multiple fertilization. The lack of evidence suggesting an XX-XY mosaicism superimposed on the mosaicism found in our cases is additional evidence against this hypothesis since this would be expected in 50% of the cases.

The majority of reported chromosomal mosaics have been attributed to postfertilization mitotic errors and the mosaicism noted in the Cornelia de Lange syndrome may originate in this period. This hypothesis would appear to be less attractive in that the influence of an environmental factor is implied, with only a segment of the cell population undergoing chromosomal rearrangement, and the resultant rearrangements would appear to be determined by chance. This would be true if the teratogenic agent was nonselective with respect to the chromosomes that are involved in the rearrangement. The possibility must be considered, however, that certain agents, particularly viruses, by virtue of the information encoded within their nucleic acids may effect characteristic chromosomal rearrangements within a proportion of the cell population by alteration of relevant biosynthetic mechanisms. This possibility, we believe, is deserving of much more attention.

The familial incidence recorded by the Wisconsin group[7, 9] is difficult to correlate with our findings of chromosomal mosaicism in the de Lange syndrome. At least five families are known with more than one affected member and this group has stated that this finding is compatible with autosomal recessive inheritance. We believe that there is insufficient evidence on which to base firm conclusions as to etiology, but a number of possibilities may be advanced to explain the infrequent familial incidence. One possibility is that a euploid reciprocal translocation is transmitted and sporadic multiple fertilizations occur involving structurally heterozygous products of oogenesis or spermatogenesis. A second possibility is that the chromosomal rearrangements are under genic control as has been described in meiosis in Drosophila[21] and mitosis in man.[22] The pedigree of Case 1 is interesting in this respect in that 4/9 members are retarded, with one, the propositus, showing a typical de Lange syndrome. One would suspect that a gene (or genes) has been transmitted which predisposes to chromosomal breakage and rearrangement, but the resultant rearrangements are determined, in part, by chance or other unknown factors. However, this instability may be limited to a few chromosomes within the cells of an individual zygote by other genic influences, resulting in simple deletions or translocations as noted in Cases 1 and 2. Moreover, this chromosomal instability may result in the continuous formation of characteristically aberrant cells through mitotic errors, and these cells may not proliferate as rapidly as euploid cells or may survive for a limited period without dividing. This hypothesis is compatible with our findings on the cultural characteristics of de Lange cells, and possibly with the idea of an autosomal recessive determinant, advanced by the Wisconsin group. The aberrant cell population would not originate from isolated meiotic or mitotic errors and could not be accurately classified as an aberrant stem line. A third possible etiological factor may be the presence of a latent long-term viral infection which becomes manifest by inducing characteristic chromosomal aberrations within a proportion of the total cell population of developing zygotes.

Many of the questions raised by the results of this investigation can be answered only by more complete studies. The fact that mosaicism has not been demonstrated in Case 3 does not seriously detract from our interpretations, for mosaicism may not be present in all cell populations. We are completing cytogenetic studies of the propositi and their families as well as studies on the cultural characteristics of de Lange cells. The latter investigations may do much to establish some order in the apparently conflicting reports from various sources.

SUMMARY

The clinical and cytogenetic findings in two typical cases and one *forme fruste* of the Cornelia de Lange syndrome are described. A euploid/aberrant double stem line mosaicism was found in the two typical cases. Our findings suggest that a partial monosomy for a segment of the long arm of a Gr(6-12)X chromosome (probably chromosome 11) in the second stem line results from a simple deletion, as in one case, or by loss of chromosomal material during the process of reciprocal translocation as in the second case. This partial monosomy may have a lethal effect if present in all cell populations and the ameliorating effect of a normal cell population permits survival of the organism. The aberrant stem line may not be present in all tissues or may not proliferate under certain cultural conditions, so that mosaicism was not detected in many of the earlier investigations and the *forme fruste* of our series. Additional evidence is needed to support our contention that the de Lange syndrome is the phenotypic expression of chromosomal mosaicism. The etiology of chromosomal mosaicism is discussed.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

THE DREADED GASEOUS INFECTION

If the dreaded gaseous infection has made itself known, by reason of subcutaneous emphysema, brownish discolouration of the skin, bubbles of gas in the brownish red discharge or by the characteristic odour of this, the wound must be more widely opened and we have also employed circular incisions of the skin and subcutaneous tissues above the areas of discolouration to stop the spread of the infection. The wounds are now irrigated with hydrogen peroxide twice a day and at the same time oxygen is injected with a hollow needle above and below, heed being taken that this procedure drives the infection towards the original wound. Between dressings, gauze moistened with hydrogen peroxide, is applied. Often the temperature is not above 100°, but the pulse is always much quickened. We have not tried Wright's treatment of such wounds with hypertonic saline and sodium citrate. For the most part we have been successful in saving the life of the patient and in saving the limb, though, at times, at the expense of, what one might call, cruel deforming operations. From the point of view of prognosis, the cases fall into two broad classes, a less severe where the infection is only in the directly subcutaneous tissues and a second one where the outlook is much more grave, in which the muscles are involved. Professor Wineberg of the Pasteur Institute, Paris, visited us to introduce his anti-serum—made by inoculating horses with increasing doses of the organism. He hopes soon to supply all the allied armies with a sufficient quantity for a prophylactic dose after every wound. So far we cannot say anything definite as to the success of such treatment with serum. Unhappily in the case now to be cited, no treatment could have had any avail.

L.C., a Belgian soldier, was admitted to the *"ambulance de Dr. Depage"* at ten o'clock on the morning of April 20. He had been wounded in action near Dixmude twenty-three hours before by a rifle bullet which had entered the outer and upper part of the left thigh and had passed both thighs grazing the scrotum *en route*. Neither femur was touched. The ordinary field dressing had been applied at once and had been changed at 9 p.m. on the 19. Antitetanic serum had been given. On admission the patient appeared gravely ill and very toxic. His temperature was 101.4° and pulse 112 and weak. The respirations were increased. He was vomiting thin brownish liquid. The left thigh was not swollen and showed nothing beyond the wounds of entrance and exit. The right, however, showed a few brownish patches just below the inguinal ligament and was enormously swollen and "crackled" beneath the fingers, below as well as above the level of the track of the bullet. This subcutaneous emphysema extended up as far as the right axilla and over the front of the abdomen but there was no discolouration in these regions. On pressing the right thigh very foul smelling brownish discharge came out from both wounds together with bubbles of gas. The thigh was tympanitic on percussion. Amputation would have been useless. A few incisions were made just above the patchy skin and gas escaped under considerable pressure. The subcutaneous fat was of a greyish green hue. Anti-serum was given intravenously and injected into these incisions—altogether sixteen cubic centimetres. The thigh was dressed with gauze soaked in the serum. The patient was put to bed and morphia administered. We saw him later at 3 p.m. when he was still vomiting. There was very extensive discolouration by this time over the abdomen, chest and even down the arms as far as the wrists. The pigmentation had also extended down the right leg. The odour in the room we shall never forget. The patient was kept under morphia and died at 7 p.m., just thirty-two hours after a rifle bullet wound in the thigh. It was extremely interesting to us that the left thigh, in which the bullet had first entered, showed no infection. Obviously the organism must have been on the clothes or the skin of the inside of the thighs or scrotum. I believe the bacillus is often found in the fæces. At post mortem less than two hours after death the organism was recovered from blood taken from all parts of the body. In the right thigh was found a large *"phlegmon gazeux"*. There was no peritonitis but marked œdema and active congestion of the mucous membrane of the bowel. Had he come to us sooner, something might have been done.—T. A. Malloch, *Canad. Med. Ass. J.*, 5: 735, 1915.