occur at random. In human cells transformed with SV40 virus,[2] and in hamster cells infected with herpes simplex virus,[1] some chromosomes were affected more than others, and specific loci on certain chromosomes were characteristically liable to changes.

Obviously more data are required before it can be established with certainty whether viruses do cause chromosomal alterations *in vivo* and, if so, whether the damaged cells can complete mitosis with viruses assuming a mutagenic role.

SUMMARY

No significant difference in the number of chromosomal abnormalities in circulating leukocytes was observed in children with mumps, chickenpox, measles, and in others following measles vaccination, as compared with abnormalities noted in a control group. Of a total of 622 cells examined in metaphase, only 12 showed abnormalities (gaps and breaks), and of these abnormalities, five were seen in the 12 controls.

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Cardiac Myoglobin in Myoglobinuria

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ABSTRACT

Studies were undertaken to determine whether significant abnormalities were present in cardiac myoglobin in a case of primary paroxysmal myoglobinuria.

No qualitative abnormality was found in cardiac myoglobin from a patient with myoglobinuria, compared to normal controls, using urea starch gel electrophoresis. Quantitative analysis, however, revealed depletion of cardiac myoglobin in the patient with myoglobinuria.

It is considered that the basic defect in primary paroxysmal myoglobinuria is not related to myoglobin *per se*, and although evidence is lacking, an autoimmune process, as encountered in some of the hemolytic anemias, may be involved in this condition.

SOMMAIRE

On a cherché à déterminer s'il y avait présence d'anomalies notables de la myoglobine cardiaque dans un cas de myoglobinurie paroxystique primaire.

Par rapport à des témoins, on n'a découvert aucune anomalie qualitative de la myoglobine cardiaque chez un malade présentant une myoglobinurie. La méthode d'analyse utilisait l'électrophorèse au gel d'amidon additionné d'urée. Par contre, l'analyse quantitative a révélé une déplétion prononcée de la myoglobine cardiaque chez ce malade.

On estime que le trouble profond dans la myoglobinurie paroxystique primaire n'est pas attribuable à la myoglobine en soi, et, bien qu'on n'en aît aucune preuve, un processus auto-immun, qu'on observe notamment dans les formes d'anémies hémolytiques, peut jouer un rôle dans la pathologie qui nous occupe.

PRIMARY paroxysmal myoglobinuria is a condition of unknown etiology, first described by Meyer-Betz[1] in 1911 and since reported with increasing frequency.

The pathological process involves striated muscle which suddenly undergoes lysis, releasing large amounts of myoglobin into the circulation. Bowden *et al.*[2] termed this condition acute recurrent rhabdomyolysis, and reported a number of cases from the Research Institute of The Hospital for Sick Children, Toronto, showing strong genetic relationships. Hed[3] and Wheby and Miller[4] also described a number of cases with familial tendencies.

Other authors, such as Bailie[7] and Perkoff,[5, 6] have suggested that abnormalities in myoglobin exist in the myoglobinurias. With this observation in mind, investigations were undertaken to determine whether abnormalities existed in cardiac myoglobin of autopsy material from a case of acute paroxysmal myoglobinuria.

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CASE HISTORY

A 45-year-old dye-setter was admitted to the Wellesley Hospital, Toronto, on March 20, 1965, with generalized muscular aches, dark brown urine and a low urinary output. He had been transferred from a hospital in Orillia on the same day. Two weeks before admission he had developed an upper respiratory tract infection with stuffiness in his head, headache and general malaise, but no cough. His wife had similar symptoms. On March 18, he became quite short of breath and developed severe aches and pains in his abdomen and extremities which were aggravated by body movement. His urine had been dark for 24 hours. Tetracycline therapy was begun at home and he was admitted to hospital in Orillia on March 19.

Ever since childhood, following exercise, he had noted aching joints and muscles, along with weakness. He sometimes had to be carried home from school. In the Army during World War II he was investigated for these complaints and was thought to have methemoglobinemia. Attacks of muscular aching would come on suddenly following exertion, as often as five to six times per year. They usually lasted 24-48 hours, with spontaneous recovery. His urine would become dark and scanty in amount. The last attack was one month prior to admission.

Laboratory studies revealed the following: His hemoglobin was 15.8 g. % and his leukocyte count was 16,700 per c.mm. with a normal differential count. The serum potassium was 5.7 and the CO_2 content was 16.9 mEq./l.

After admission to hospital despite restriction of fluid intake and chemotherapy with an oral chelating agent (Kayexalate) and an osmotic diuretic (Osmitrol), the patient developed increasingly severe oliguria. Peritoneal dialysis was carried out on March 23, without clinical improvement, and the patient died suddenly, 12 hours after dialysis, following a generalized clonic seizure.

EXPERIMENTAL PROCEDURES

Cardiac muscle was obtained from autopsy material from young adults who had died in automobile accidents, and from the above patient with myoglobinuria. The muscle was stored at —20° C. for 48 hours before extraction.

Cardiac myoglobin was extracted using essentially the method of Ginger, Wilson and Schweigert.[8]

(a) *Preparation of cardiac myoglobin*: 50 g. of cardiac muscle was "blended" (Onmimix) in 50 ml. of distilled water and allowed to remain in the cold room (2°-5° C.) overnight. The supernatant obtained after centrifugation was adjusted to pH 7.0 to which was added 12.5 ml. of saturated basic lead acetate. The precipitate was removed by centrifugation. The phosphate ion concentrate was adjusted to 3M (pH 6.6) by the addition of monohydrogen and dihydrogen potassium phosphate. The precipitate was removed by centrifugation and the supernatant was dialysed against eight changes of distilled water. The material from the dialysis bag was brought to 85% saturation with ammonium sulfate. The precipitate was collected by centrifugation and the supernatant, which contained the myoglobin in ammonium sulfate, was dialysed against 10 changes of distilled water. The contents of the dialysis bag were lyophilized in some cases. However, this had the effect of rendering the myoglobin insoluble. For column chromatography the contents of the bag were used without dialysis.

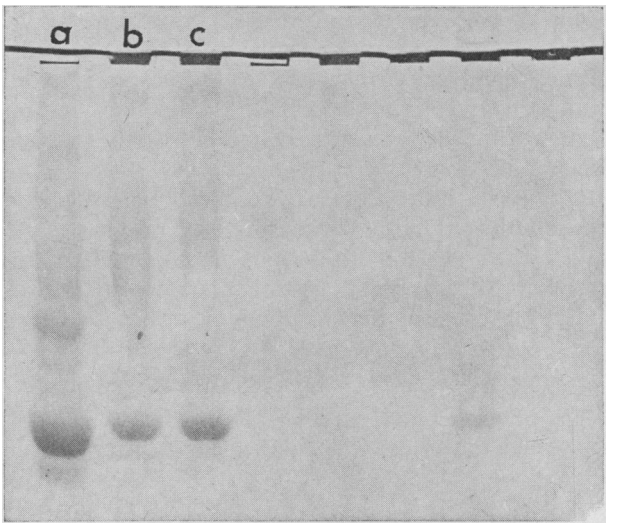

Fig. 1.—Urea-starch gel at pH 3.4 showing (from left to right) (a) cardiac myoglobin as prepared; (b) and (c) DEAE chromatography of myoglobin: (b) fraction 82-84; (c) fraction 85-87.

(b) *Column Chromatography*: Myoglobin was chromatographed on DEAE-cellulose columns equilibrated with 0.005 M tris buffer (pH 7.85); myoglobin, 13 mg., was applied to a column 2.5 x 18 cm. and eluted according to the method described by Perkoff *et al.*[9]

(c) *Starch Gel Electrophoresis*: The vertical starch gel method described by Smithies[10] was used throughout. Tris-borate gels were used at pH 8.6. After slicing, half the gel was stained with benzidine and half with amido black. Urea starch gels (pH 3.4) were prepared by the method of Smithies, Connell and Dixon.[11]

(d) *Protein Determination*: The Lowry modification of the Folin method was used.[12]

RESULTS

Myoglobin Extracted from Normal Heart Muscle

The electrophoretic findings on a typical preparation are shown in Fig. 1, a photograph of a urea starch gel. In slot (a) the supernatant, after ammonium sulfate precipitation, shows one heavy band and at least three light bands.

When such a myoglobin preparation is chromatographed on DEAE-cellulose, as described above, one major component is present, as seen in slots (b) and (c) of Fig. 1. This material was shown not to be hemoglobin but to stain positively with benzidine after electrophoresis in tris-borate starch

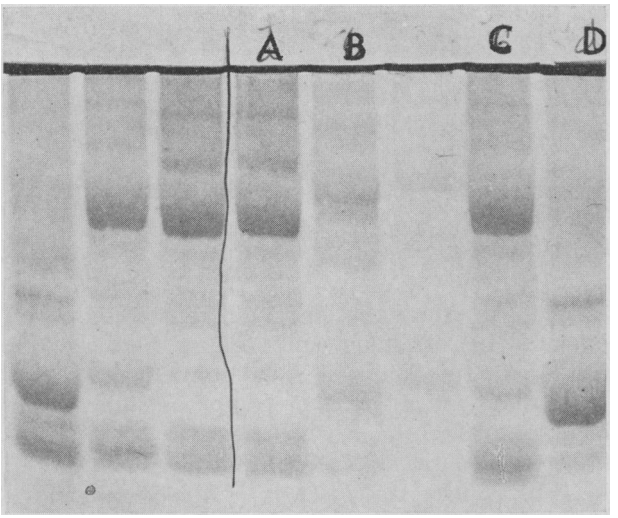

Fig. 2.—Urea-starch gel, pH 3.4, of cardiac myoglobins. (a) Fraction precipitated by 85% ammonium sulfate from a case of paroxysmal myoglobinuria. (b) Supernatant from (a). (c) Fraction precipitated by 85% ammonium sulfate from normal cardiac muscle. (d) Supernatant from (c).

gel at pH 8.6. A soret band was found at 417 mμ. and a protein peak at 280 mμ.

Myoglobin Extracted from a Case of Myoglobinuria

In Fig. 2, a urea-starch gel preparation, myoglobin extracted from normal cardiac muscle, is contrasted with the electrophoretic findings in the case of myoglobinuria described above. Comparing the patterns found in slots A and B with those in slots C and D, it can be seen that they are qualitatively similar. The major myoglobin band, M1, in slot D is also present in slot B.

When a comparison was made of the amounts of myoglobin extracted from normal heart muscle and cardiac muscle from the case of myoglobinuria, the following results were obtained: After 28 different extractions from as many independent samples of heart muscle, we obtained between 150 and 200 mg. of myoglobin per 100 g. of tissue. In the case of myoglobinuria, we obtained only 10 to 20 mg. of myoglobin per 100 g. of cardiac muscle.

Myoglobin Isolated from the Urine in a Case of Myoglobinuria

Urine was collected from a neonate who developed myoglobinuria following a prolonged convulsive episode. The baby survived and its myoglobinuria has subsequently cleared.

The urine was dialysed against six changes of distilled water and brought to 85% saturation with ammonium sulfate by addition of solid ammonium sulfate. The precipitate was removed by centrifugation and the supernatant was dialysed against 10 changes of distilled water. The myoglobin solution was lyophilized.

The material isolated from the urine behaved like normal myoglobin on starch gel electrophoresis.

DISCUSSION

Myoglobin extracted by the method described above yields distinct zone electrophoretic patterns. The major component was identified as myoglobin by column chromatographic and starch gel characteristics, and by the presence of a soret band at 417 mμ.

Myoglobin extracted from cardiac autopsy material in a case of paroxysmal myoglobinuria showed no abnormality in urea-starch gel pattern as compared with the normal controls, i.e. the major and most of the minor components were present. The basic defect in this condition most probably is not related to myoglobin *per se* but to the other muscle components. Although evidence is lacking, some autoimmune process as seen in some of the hemolytic anemias with hemolysis may be involved here.

As demonstrated quantitatively, acute myoglobin depletion of muscle tissue, including myocardium, occurs in myoglobinuria. The resultant hypomyoglobinosis gives rise to relative cellular anoxia with serious and often fatal consequences.

The fact that we were able to obtain only minute amounts of myoglobin from cardiac muscle in our case of myoglobinuria is direct confirmation of data presented by Bowden *et al.*[2] These authors consider that myoglobinuria can best be thought of as recurrent rhabdomyolysis, a term which they feel more correctly describes the pathological condition.

The isolation of myoglobin from urine in a neonate with myoglobinuria showed essentially the same pattern on urea-starch gel as that extracted from cardiac muscle.

SUMMARY

Investigation of a case of primary paroxysmal myoglobinuria revealed no qualitative abnormality, in cardiac myoglobin, as compared to that of normal controls, on urea-starch gel zone electrophoresis.

Acute depletion of cardiac myoglobin in patients with myoglobinuria occurs, as demonstrated quantitatively, with a resultant state of hypomyoglobinosis.

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