whether Met-Hb was actually absent, because we ourselves would more than once have failed to find it if we had not made special efforts. In such cases one must use for the spectroscopic test: (1) a solution of blood in distilled water in the proportion of 1:20, 1:10, or even 1:5; (2) a strong light to develop the spectrum. It often happens, in fact, that Met-Hb, when present in small quantities, does not give the characteristic band in the red if the solution of the blood for spectroscopic examination is too diluted.

The quantitative measurement of Met-Hb by the Dubost method has given us yields from 25–50% of the total haemoglobin (Vaftochilary family), 18–40% for the Melaniarides, and 10–52.5% for the cases of Zacoboulos. It was always intracellular and never extracellular, and discoverable *in vivo* in the ear lobe.

As regards other blood examinations, occasionally we find a slight increase in red and white blood cells, the haemoglobin is either normal or slightly subnormal, the cells normal, and other blood constituents all normal, like the W.R. Urine analysis has also usually been normal.

We have failed to find any correlation whatsoever between this methaemoglobinaemia and blood groups, including the Rh factor. In a certain number of our patients we found a decrease below 0.40 mg. ascorbic acid per 100 ml. of blood. This C-hypoavitaminosis was confirmed by the saturation method.

C. Prognosis

The prognosis of H.M.C. is good, since most of the patients reach an advanced age.

D. Treatment

Although convinced of the hereditary nature of the disease, we have also, however, tried the therapeutic means employed in other cyanoses—ascorbic acid and methylene blue—by intravenous injections or orally. The favourable influence of these drugs on the cyanosis as well as on the subjective troubles of the patients and the sometimes spectacular reduction of methaemoglobin to haemoglobin are only temporary, and continuous oral administration of ascorbic acid is necessary. It is interesting, by the way, to note that the patients themselves felt the favourable effect of lemon and orange juice and other foods rich in ascorbic acid.

Although it is true that the light and medium cases of methaemoglobinaemia are symptomless, the cyanosis in itself is nevertheless not very pleasant, especially for young girls. Thus one sees them submitting to an intravenous injection of methylene blue or ascorbic acid (300 to 500 mg.) in order to obtain, usually at the end of 10 minutes, a decoloration which may last 10 to 15 days, and which is made to last by absorbing 10 to 30 mg. ascorbic acid daily if they do not prefer an injection every 10 or 15 days.

Summary

A new genealogical tree of hereditary methaemoglobinaemic cyanosis (H.M.C.) of five generations, comprising 85 members and including 10 born cyanotics, is described.

H.M.C., also known as congenital or familial cyanosis, or methaemoglobinaemic idiopathic cyanosis, is a distinct clinical entity characterized by the presence of methaemoglobin in the blood, and the absence of clubbing of the fingers. It exists from birth and is inherited by both sexes, with a preponderance in men.

It is thus quite distinct from cyanosis due to congenital heart disease or arteriovenous shunt, to polycythaemia, or to drug intoxication.

Although 42 out of the 92 described cases are in Greeks, the condition has been reported from all continents, so far only in white men.

It is a pleasant duty to express our gratitude and thanks to Mr. Constantine Georgopoulos, president of the Hellenic Red

Cross, for the encouragement and support which he gave us in the pursuit of our investigations; and also to Professor D. Chondros for his learned advice and for the loan of the precious spectroscope, of which he deprived himself in order that we might make our measurements of methaemoglobin.

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METHAEMOGLOBINAEMIA DUE TO NITRATES IN WELL-WATER

BY

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Since Comly (1945) reported two cases of cyanosis due to methaemoglobinaemia in young infants caused by the ingestion of nitrates in well-water, numerous cases have been reported from Canada and the U.S.A., a few from Belgium, and some from other countries. Ewing and Mayon-White (1951) reported cases found in a rural area of Suffolk, which were the first recorded in the United Kingdom. It is believed that the present case is the first to be detected in Northern Ireland, although the circumstances necessary for the production of the condition must be relatively common in a predominantly rural community such as this.

Case Report

This infant, a female, was born by normal spontaneous delivery at full term; she cried quickly and gave no trouble in the immediate post-natal period. She was breast-fed for three weeks only, and then weaned on account of "insufficient milk." She was put on to feeds of full-cream dried milk, on which she gained weight. One week after receiving the artificial feeds she began to vomit two or three times a day, generally one to two hours after a feed, quite copiously but not very forcefully. She appeared to be in pain before the vomit. Citrate tablets given in the feeds seemed to reduce the vomiting for a week, but it then re-occurred, and for five days before her attendance at the outpatient department it had been severe. The bowels were opened once or twice a day, the stools being greenish. On the day before attending hospital she was noticed to be blue, almost black, about the lips and finger-tips.

On examination (June 29, 1951) the infant was seen to be well nourished and energetic. There was generalized slate-blue cyanosis, especially over the lips and fingers. The mouth and throat were healthy. No abnormal signs could be detected in the heart or lungs. The abdomen was normal, the spleen could not be palpated, and no pyloric tumour was felt. The association of marked cyanosis with the generally well-nourished appearance of the infant and the absence of signs in the heart or lungs led to a tentative diagnosis of enterogenous cyanosis. Inquiry revealed that the family drew their water from a well on their own farm. The child was accordingly admitted for confirmation of the diagnosis and for treatment.

Investigations.—Unfortunately it was not possible to carry out a blood examination until the following morning, by

which time she had had several feeds in hospital. A specimen of venous blood showed the typical "chocolate" appearance of the methaemoglobinaemia to the naked eye, and, although the clinical cyanosis had decreased even in the 12 hours since admission (without treatment), methaemoglobin was easily detected spectroscopically in a dilution of 1 in 10. The peripheral blood picture showed: Haemoglobin, 76% (Haldane); red cells, 3,580,000; normoblasts, 4 per 100 leucocytes; and polychromasia. Two specimens of fasting gastric juice were taken; the first had a pH of 3.8 with no free acid, and gave a growth of coliform organisms on culture; the second had a pH of 3.3, no free acid, and was sterile. A sample of the well-water was analysed for nitrate; the following result was obtained: 116 parts per million (as NO_3); 26.2 p.p.m. as nitrogen. This was reported to the divisional medical officer, who arranged for bacteriological examination of the well-water, and found it to be "unsatisfactory," with 50 coliform organisms of faecal origin per 100 ml.

Progress and Treatment.—As soon as blood samples had been obtained, treatment was started with 50 mg. of ascorbic acid in each four-hourly feed. Cyanosis, which had begun to fade even before treatment, had completely disappeared by the fourth day. The infant gained weight steadily—10 oz. (283 g.) in five days—and was discharged home on July 5, six days after admission, with no signs or symptoms. Arrangements were made by the family to obtain water from a branch of the main Belfast water supply, which passes close to their home. The infant apparently did not feed well after discharge, was irritable, and lost weight—1 lb. (454 g.) in 14 days. The stools became more frequent ten days after discharge and vomiting started on July 18. The child was readmitted, and was found to be very dehydrated and lethargic. There was no cyanosis on this occasion. The condition was thought to be an infective gastro-enteritis, and as soon as the dehydration had been corrected the infant was transferred to the Northern Ireland Fever Hospital, where she had a stormy course, but eventually recovered, being discharged on September 26. No cyanosis was noted while in that hospital. Her progress since then has been uneventful, and she has had no feeding difficulty. Water is still being obtained from a neighbouring farm which has a piped supply.

Discussion

When an infant in the first few weeks of life is found to be cyanosed, the suspicion of congenital cardiac lesions or of some form of respiratory obstruction is likely to arise, and such causes do in fact account for most cases. It is of interest in this connection that Donahoe (1949) states that all five of the cases which he reports had been treated by x rays for presumed enlargement of the thymus before the true cause of their cyanosis was established.

Methaemoglobinaemia sufficient to cause obvious cyanosis may be produced in a variety of ways, none of which are really common. Apart from cases of familial idiopathic methaemoglobinaemia (Breakey *et al.*, 1951), in which there is an intrinsic disturbance of the normal enzyme reactions in the erythrocytes, the condition may be caused by a wide variety of extrinsic factors acting on the erythrocytes, either infections or chemical poisons. These may be divided, according to Ferrant (1946), into those producing plasma methaemoglobinaemia—for example, blackwater fever, sepsis (Winckel's disease), etc.—and those giving cellular methaemoglobinaemia, including so-called "enterogenous cyanosis" and a wide range of drugs and other chemicals. The latter not only may be ingested, but in certain cases may be absorbed through the unbroken skin of infants—for example, aniline dyes used for marking napkins or naphthalene moth crystals. Walliker and Baxter (1949) list a large number of these causes and describe illustrative cases.

Comly (1945) is generally recognized as having reported the first cases of methaemoglobinaemia in infants that could be shown to be due to the use of well-water with a high nitrate content in the preparation of artificial feeds. Since then many cases have been added to the literature; those

published up to 1950 are reviewed by Lecks (1950). He also describes the clinical appearance of these infants, showing cyanosis, gastro-intestinal upsets, and developing lethargy, progressing to apathy with air-hunger and convulsions in severe cases. There are characteristically few physical signs apart from the cyanosis, and this latter is typically of a "slate-grey blueness," distinguishable from that found in congenital heart disease.

In all cases the water supply has been from a shallow well, easily contaminated, or occasionally a deep well with defective casing. The condition is most likely to occur in infants fed on dried milks, less often on evaporated milk, and least on mixtures of cows' milk and water; this is because the proportion of contaminated water is naturally higher in the first two, and therefore the total amount of nitrate ingested is greater. However, if the cows use the same water supply their milk may contain enough nitrate to be dangerous, and one case is reported by Donahoe (1949) in which cyanosis developed in a breast-fed infant whose mother was drinking contaminated water, the cyanosis disappearing when the water supply was changed.

The actual concentration of nitrate found has varied from 15 to 250 or more parts per million of nitrate nitrogen, usually with traces of nitrite as well. Weart (1948) and Hoather (1951) attempted to relate the nitrate content to the degree of bacterial contamination, but, although most of the samples of water were unsatisfactory from the bacteriological standpoint, no very close correlation could be found. Boiling the water does not affect the nitrate content and therefore it cannot be made safe for use in preparing feeds in this way. Methods have been devised for removing the nitrates (Krueger, 1949), but in most cases it will be more satisfactory to obtain an alternative supply of water.

Adults do not appear ever to be affected by the water, and can drink it with impunity so far as the nitrate content is concerned. Several theories have been advanced to account for the exclusive appearance in infants: Cornblath and Hartmann (1948) found that the gastric juice of affected infants had a low free acid, with pH 4 or higher; this permitted the growth of nitrate-splitting organisms higher up the gastro-intestinal tract than is possible in adults, the nitrate thus formed being absorbed in the small intestine. Ferrant (1946) holds that nitrate is normally split to nitrite and then to ammonia before absorption takes place, but that if excess nitrate is given this change cannot take place quickly enough and nitrite is absorbed in significant quantity. This is also held by Medovy *et al.* (1947). Another possible factor is that nitrite forms methaemoglobin more easily with "foetal" haemoglobin than with the adult form.

Treatment is fortunately comparatively simple, and is rapidly effective in all but the most severe cases. In mild degrees of the condition, merely removing the source of nitrate will permit a spontaneous cure in a few days: the case reported above showed clinical improvement within 12 hours of admission to hospital. In moderately severe cases ascorbic acid by mouth or intravenously will produce a cure in 12 to 24 hours. In severe cases methylene blue, given intravenously in 1% solution, 1–2 mg. per kg., will produce clinical cure within 30 minutes.

Relapse can be prevented by feeding the infant on acidified (lactic acid) feeds, but, as the water is likely to be bacteriologically suspect, it is preferable to obtain it from an alternative source.

Summary

A case of cyanosis in a month-old infant, due to methaemoglobinaemia caused by the presence of nitrate in well-water used for preparing feeds, is reported from Northern Ireland.

The infant made a rapid recovery on treatment with ascorbic acid.

It is suggested that similar cases may arise more often than has previously been recognized in rural

areas. Such infants may die if the cyanosis is attributed to congenital heart disease and treatment is not given.

My thanks are due to Mr. D. W. Neill for carrying out the spectroscopic examination of the blood and for estimating the nitrate content of the water.

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CUTANEOUS REACTIONS TO INJECTION OF PROGESTERONE SOLUTIONS INTO THE SKIN

BY

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It has been noticed that the intradermal injection of progesterone solution causes a complex reaction in the skin—an immediate and transitory “triple response” (Lewis, 1927), followed after about a week by a localized follicular eczema, which on subsiding leaves a pigmented patch. It is known that in different circumstances oestradiol or testosterone may increase skin pigmentation; and the present observations are perhaps mainly of interest in that they provide a further example of such an effect of a sex hormone.

Experimental Procedure and Results

Progesterone solutions were injected into the skin of 29 medical students, 10 being women, and into my own skin. Each received two intradermal injections at points a few centimetres apart on the anterior surface of the forearm; one was of “progestin,” containing 1 mg. of progesterone per ml. in ethyl oleate, the other of progestin, 25 mg. per ml. In each case 0.04 ml. was injected, but measurement showed that about half of the oily solution rapidly escaped through the track of the needle. The quantities of progesterone that remained in the skin were therefore about 0.02 and 0.5 mg., and of ethyl oleate 0.02 ml.

In every case a stinging sensation and a widespread flare developed within a minute or two. Wheal formation at this stage was minimal, and (as shown by subsequent tests on one subject) the size of this initial reaction depended on the volume of solution injected, of whichever strength, rather than on the amount of progesterone it contained.

This reaction subsided during the first day, and for the next week many of the subjects showed little evidence of the injection. Then from about the seventh day onwards a further reaction was seen in all but 5 of the 30 subjects. It looked like a small patch of follicular eczema, but it was only mildly irritating. Each “follicle” was the centre of a minute area of brown pigmentation; when the blood was pressed out of the distended minute vessels by means of a microscope slide the pigmentation was more obvious. The pigment was not identified, but throughout all stages of its development and fading it was indistinguishable in colour

from that of sun tan. During this period in the summer many of the subjects developed a moderate natural sun tan which overlapped the experimental areas.

When the experimental reaction was at its height, at about the fourteenth day, 13 subjects showed at the site of the injection of the larger amount of progesterone a patch of pigmentation which was judged to be equivalent to a marked sun tan and a further 8 showed one equal to a mild sun tan; the corresponding figures for the smaller concentration of progesterone were 2 and 16 respectively. Although these figures are the result of subjective judgment, there is no doubt that, at this stage, in many cases the pigmentary reaction to the more concentrated solution was greater than that to the less concentrated. Only 4 of the 30 subjects showed no pigmentation whatever.

The occurrence of pigmentation of any degree was distributed evenly among those with fair, medium, or dark hair and skins, but the densest was seen in dark (European) subjects. There was no preponderant incidence in either sex. The pigmentation persisted in most cases for some weeks, although the eczema-like reaction subsided during the next few days.

Then in nine subjects (four men and five women) an unexpected reaction occurred. During the third week after the injection the site, particularly that at which the more concentrated solution had been introduced, became first itchy and then “inflamed,” with eczematous follicles and wheals. The surface desquamated and the whole area of about 5-cm. diameter became apparently oedematous and pervaded by the brown pigment. Although half the women showed this more severe reaction, as compared with one-fifth of the men, there was no significant correlation with the phase of the menstrual cycle. The most severe reaction was in a woman. Some subjects had an intense irritation, and the application of adhesive tape to hold dressings in place caused subsidiary foci of reaction. Although the students had by now dispersed and the later stages of the reactions could not be watched personally, it seems that in no case did any infection of the areas occur.

Mepyramine hydrogen maleate (“anthisan”) cream gave some relief, but usually was not completely effective. The reactions subsided, in some cases with recrudescences, during the next week or month. The pigmentation tended to fade a little more slowly than the other manifestations.

Further similar injections of the oily medium alone (ethyl oleate) were made into my skin. It was apparent that the initial triple response could be caused by injection of the medium alone. The later eczematous reaction appeared, but in smaller degree, and it was very doubtful whether any pigmentation resulted. Slight pigmentation, however, followed intradermal injection of normal saline. I did not suffer any severe reaction to either the progestin or the ethyl oleate injections; but, inasmuch as the other reactions appeared to be due partly to the medium, and some of the other subjects had experienced unpleasant reactions, it was not felt justifiable to repeat the injections with the medium on the other subjects.

Repetition of pairs of injections, of progestin and ethyl oleate, in my skin during a period of several months gave gradually diminishing responses to both.

Discussion

The evidence suggests that the initial triple response and the eczematous reaction of the second week are non-specific responses to the injection of the oily medium, ethyl oleate. The greater intensity of pigmentation at the site of injection of the concentrated solution, and possibly the whole of the later severe reaction that occurred in 4 of the 20 men and in 5 of the 10 women, seem to depend on the presence of progesterone in the injected solution. Some examples are known of similar effects of sex hormones. The localized hyperpigmentation of pregnancy can be imitated by oral administration of oestrogens (Davis *et al.*, 1945), but the possible participation during pregnancy of other hormones such as progesterone does not seem to