one year became resistant to both tolbutamide and metformin and required insulin for control.

Comment

In our small series the type of patient who appeared to respond best was over the age of 40, had no tendency to severe ketosis, and was not suffering from " pancreatic diabetes "—the type of case, in fact, that may well respond to sulphonylureas. Metformin can therefore be considered in such cases if there is complete or partial resistance to the sulphonylurea derivatives. Two of our cases were resistant to tolbutamide but responded satisfactorily to metformin.

Although in this trial a response to metformin occurred initially in some adults of all ages, this did not persist in those under 40. It is possible that the drug may sometimes be successful under the age of 40 if there is no tendency to ketosis, but it seems likely that resistance may develop subsequently. When partial resistance to metformin develops, as it did in four cases at the end of six months, a satisfactory response may occur when tolbutamide is added to the metformin. In one of these cases, that of a man aged 21, resistance to both substances finally developed at the end of one year, and insulin became necessary.

Metformin seemed to have no place in the treatment of " pancreatic diabetes." Our three cases were completely resistant to this treatment and required insulin, small doses of 10–20 units being effective in two.

A case of steroid diabetes which responded satisfactorily to metformin deserves special comment. A woman of 47 suffering from rheumatoid arthritis for many years required 15 mg. of prednisolone daily to control her symptoms. Periodic urine tests revealed no glycosuria until six years after beginning treatment, when she had intermittent glycosuria, with blood sugar of 87 mg./100 ml. fasting and 256 mg./100 ml. two hours after food. Because of the activity of the rheumatoid arthritis the same dose of prednisolone was continued and, despite a lowered carbohydrate intake, her glycosuria persisted. She responded very satisfactorily to metformin, and eight months later remained sugar-free although still continuing with the same dose of prednisolone.

Side-effects in this series were troublesome enough to cause the drug to be stopped in two cases, one having nausea and occasional vomiting, and the other diarrhoea. Other patients had slight anorexia, but when given their metformin tablets with the meals this tendency disappeared. Diarrhoea appeared in two other cases when metformin was given in full doses of 3 g. daily, but the symptom disappeared when the dose was reduced to 1.5 g. None of our patients showed evidence of liver or kidney damage. One patient periodically passed in the urine small uric acid calculi 2–3 mm. in diameter while on metformin therapy. These produced no symptoms. Their relation to the treatment is uncertain.

Mode of Action of Diguanides.—The mode of action of diguanide products has not been definitely determined. There is evidence to suggest that they act on the oxidative phase of carbohydrate metabolism, where they block the transfer of high-energy bonds to adenosine-diphosphate, causing a diminution of oxygen uptake and leading to a condition of anoxia, which in turn increases the rate of anaerobic glycolysis and so lowers the blood sugar (Kruger *et al.*, 1960).

Summary

A clinical trial of metformin in 39 adult cases of diabetes mellitus is described. Six months after starting treatment 14 showed a satisfactory degree of control on metformin alone. Six other cases showed some improvement when metformin was combined with either tolbutamide or insulin.

Three cases of pancreatic diabetes did not respond to metformin.

One case of steroid diabetes was satisfactorily controlled on metformin alone despite the continuation of prednisolone.

Side-effects severe enough to stop treatment occurred in only two cases.

REFERENCES

Aubertin, E., Roy, A., and Wone, Ch. (1960). *Diabète (Le Raincy)*, **8**, 5.
Azerad, E., and Lubetzki, J. (1959). *Presse méd.*, **67**, 765.
François, R., and Ruiton Ugliengo, A. (1960). *Diabète (Le Raincy)*, **8**, 98.
Frank, E. (1928). *Med. Klin.*, **24**, 563.
—— Nothmann, M., and Wagner, A. (1926). *Klin. Wschr.*, **5**, 2100.
Granville-Grossmann, K. L., Crawfurd, S., Crowley, M. F., and Bloom, A. (1959). *Brit. med. J.*, **2**, 841.
Hesse, E., and Taubmann, G. (1929). *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **142**, 290.
Kruger, F. A., Skillman, T. G., Hamwi, G. J., Grubbs, R. C., and Danforth, N. (1960). *Diabetes*, **8**, 170.
Lebon, J., Claude, R., Leutenegger, M., Galley, P., and Tricoire, J. (1961). *Diabète (Le Raincy)*, **9**, 159.
Nahum, A. (1960). Ibid., **6**, 257.
Quillon, J. (1961). Ibid., **9**, 172.
Watanabe, C. K. (1918). *J. biol. Chem.*, **33**, 253.
Werner, E. A., and Bell, J. (1922). *J. chem. Soc.*, **121**, 1790.

SIGNIFICANCE OF HAEMOGLOBINS S AND C IN GHANA

BY

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In Ghana almost 30% of the population are carriers of either haemoglobin S or C (Edington, 1959). In Accra, among 200 out-patients, the incidence of the sickle-cell trait was 17% and of the haemoglobin-C trait 10.5% (Edington and Lehmann, 1954). The high incidence and pathogenic properties of these haemoglobins cause them to be of considerable clinical importance in Ghana. Since the discovery of haemoglobin S (Pauling *et al.*, 1949) and of haemoglobin C (Itano and Neel, 1950) much knowledge has accumulated regarding their behaviour (reviewed by Zuelzer *et al.*, 1956), but the role of haemoglobin C has remained unproved (Lehmann, 1959).

Haemoglobin S has a fairly wide distribution, and carriers of the sickle-cell trait occur in frequencies of up to 40% in Africa, 30% in India, and 17% in Greece (Allison, 1954). Homozygous carriers of haemoglobin S die before puberty from the effects of sickle-cell disease (Lehmann and Raper, 1956), and survival of the gene responsible has been attributed to the protection haemoglobin S confers on heterozygous carriers against *Plasmodium falciparum* malaria. Much research has been carried out on this hypothesis and the literature has been reviewed by Allison (1957). One of the most important contributions was by Raper (1955), who

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pointed out that haemoglobin S limited the intensity of infection as judged by determination of parasite densities. It was also pointed out that the inclusion of adults in a survey would obscure results on account of the immunity they would have acquired through repeated exposure to malarial infections.

Clinical syndromes associated with haemoglobin S other than sickle-cell anaemia include fatal sickling crises in pregnant women with haemoglobin SC disease (Edington, 1957) and splenic infarction among carriers of the sickle-cell trait. Previous reports have usually associated the latter occurrence with flying in unpressurized aircraft (Rotter *et al.*, 1956), but during the present study seven examples of splenic infarction in heterozygous carriers of haemoglobin S were observed personally, of which only two occurred during air flight. Necropsy studies by McCormick (1961) support the contention that the sickle-cell trait is not so benign as previously supposed.

Haemoglobin C in high incidence is found only in West Africa, and is thought to have originated in the region of Northern Ghana (Lehmann, 1956). Spread inland seems to be limited by the River Niger (Lehmann and Nwokolo, 1959), but from the coast haemoglobin C has been carried in the past to North and South America and the West Indies through the medium of the slave trade (Walters and Lehmann, 1956). These authors stated that if haemoglobin C conferred a selective advantage against malaria similar to that exerted by haemoglobin S, then it would tend to replace the latter, since it has fewer disadvantages. The benignity of homozygous haemoglobin C disease is accepted (Smith and Krevans, 1959), and heterozygous carriers of haemoglobin C appear to be entirely asymptomatic (Kaplan *et al.*, 1953). Neel (1956) thought that the localized distribution of haemoglobin C could be interpreted as the finding to be expected for a gene of relatively recent origin with a high selective value, and Edington and Lehmann (1956) received the impression that haemoglobin C was replacing haemoglobin S in Ghana. The only previous investigation of the role of haemoglobin C in Ghana was by Edington and Laing (1957), who were unable to demonstrate any protective effect against *P. falciparum* malaria among Dagomba villagers in the Northern Territories.

The present investigation is concerned with the relationship between haemoglobins S and C and malaria in Accra.

Methods

The investigation consisted of two separate trials whose subjects were police personnel and children of police personnel in Accra. The police are recruited from all over Ghana and form a good cross-section of the whole community. Unlike the Army they receive no organized drug prophylaxis against malaria.

During the first trial, which lasted for nine months, all adult policemen reporting sick at the Military Hospital, Accra, with fever—that is, temperature of over 99° F (37.2° C.)—irrespective of the suspected cause, had thick blood films examined for malarial parasites and had venous blood taken for haemoglobin electrophoresis.

During the second trial, which extended over a period of six weeks during the wet season, thick films and finger-prick samples of oxalated blood were collected from children aged 1 to 6 years of police personnel. These children were apparently healthy and were taken at random from families attending a mass miniature radiography survey of the Accra police. Thick films were examined for *P. falciparum* and parasite densities were determined on all positive slides. Haemoglobin electrophoresis was carried out on the oxalated samples of capillary blood and sickling tests were performed where indicated.

Haemoglobin for electrophoresis was prepared in the conventional manner from venous blood, cells being washed three times before haemolysis, and the eventual solution diluted to a strength of 5 g./100 ml. However, when using oxalated capillary blood a shorter method of preparing haemoglobin solutions was used. This consisted of removing most of the blood from the oxalate bottles with a Pasteur pipette and then mixing each sample with two drops of distilled water and four drops of carbon tetrachloride in serological glass tubes. The tubes were racked and placed in a mechanical agitator for five minutes and finally centrifuged for five minutes at 3,000 r.p.m. The resultant haemoglobin solution was found to be adequate for the purpose of distinguishing between the various combinations of haemoglobins A, S, and C, the chief contaminant—albumin—travelling much faster on the electrophoretic strip than haemoglobin A. Care was taken to leave a small amount of blood in the oxalate tubes for sickling tests. Electrophoresis of haemoglobin samples was performed in hanging-strip tanks on Whatman No. 1 filter paper using barbitone buffer of *p*H 8.8. The procedure was performed in an air-conditioned room, using a current of 5 mA per strip (width 19 in.—48 cm.) for three hours at 350 V. Using two strips per tank and two tanks in parallel, it was possible to electrophorese 50 samples of blood simultaneously.

Sickling tests were performed with 2% sodium metabisulphite (Daland and Castle, 1948). These authors noted that reducing agents deteriorated on exposure to air and found that refrigeration enhanced their keeping properties. During the present investigation it was found that solutions of 2% sodium metabisulphite remained effective for seven days if kept under paraffin in a refrigerator. To combat the effect of humidity on hygroscopic sodium metabisulphite powder it was found necessary to enclose a tin of silica gel within the stock bottle, and to keep the latter in an air-conditioned room.

Thick blood films were stained with Field's stain and examined under an oil-immersion lens with ×600 magnification. Parasite densities were calculated by counting the number of parasites and leucocytes in 10 fields, assuming an average total white-cell count of 5,000/c.mm. All investigations were personally supervised, and performed with the assistance of two experienced laboratory technicians.

Results

The first trial determined the incidence of malaria according to haemoglobin type among 278 adult Ghanaian policemen reporting sick with symptoms of illness and a fever. The results are shown in Table I and reveal a significant difference in the incidence of malaria in the three main groups (χ^2 for 2 d.f. = 7.01, $P<0.05$), being highest in those with the sickle-cell trait (AS), lowest in those with the haemoglobin-C trait (AC), and intermediate in those with normal haemoglobin (AA).

The second trial determined the incidence and intensity of infection with *P. falciparum* among 840 police children, again compared according to haemoglobin type. Tables II and III summarize the results. Comparison of the incidence of positive blood films in the three main groups shows no significant difference (χ^2 for 2 d.f.=0.29, P>0.8), but the mean parasite densities of children with the haemoglobin-S and haemoglobin-C traits are significantly lower than that of children with normal haemoglobin. The mean parasite density of the AS group is lower than that of the AC, but the difference is not significant with the present sample.

The results of the first trial showed a significant difference in the frequency with which policemen of varying haemoglobin types reported sick with blood-film-positive fevers. A comparison has been made in Table IV between the incidence of haemoglobin types among adult policemen with blood-film-negative fevers (assuming these to occur equally in each haemoglobin group) and the incidence of the same haemoglobin types in apparently healthy police children. The differences are not significant and it can be inferred that the above normal incidence of malaria in adult carriers of the sickle-cell trait is not due to a sampling error. Table V shows the contrast in prognosis between homozygous haemoglobin-S and haemoglobin-C disease.

TABLE I.—*Incidence of Positive Blood Films in Adult Ghanaian Policemen Reporting Sick with Fever*

Haemoglobin Type	No.	Positive Blood Films	Negative Blood Films	% Positive Blood Films
AA	189	48	141	25·3
AS	52	19	33	36·5
AC	30	3	27	10·0
Others	7	3	4	—
Total	278	73	205	—

TABLE II.—*Incidence and Intensity of Infection with P. falciparum in 840 Police Children Aged 1-6 Years*

Haemoglobin Type	No.	%	Positive Blood Films	% Positive Blood Films	Mean Density Parasites × 10^3/c.mm.
AA	593	70·6	176	29·7	8·05
AS	123	14·7	34	27·6	1·66
AC	101	12·0	28	27·7	3·68
Others	23	2·7	—	—	—

TABLE III.—*Comparison of Mean Parasite Densities Among Accra Police Children*

Haemoglobin Type	Mean Density	Standard Deviation	Standard Error of Difference between Means	t	P
AA	8·05	14·00	1·16	5·53	<0·001
AS	1·06	2·74			
AA	8·05	14·00	1·62	2·70	<0·01
AC	3·68	6·48			
AS	1·66	2·74	1·31	1·54	>0·1
AC	3·68	6·48			

TABLE IV.—*Comparison of Incidence of Haemoglobin Types in Adult Policemen with Blood-film-negative Fevers and in Police Children in Accra*

Haemoglobin Type	No.	%	Standard Error of Difference between Percentages	t	P
AA (adults)	141	68·7	4·34	0·437	>0·7
AA (children)	593	70·6			
AS (adults) ..	33	16·1	7·15	0·195	>0·9
AS (children)	123	14·7			
AC (adults)	27	13·2	7·20	0·166	>0·9
AC (children)	101	12·0			

TABLE V.—*Incidence of Homozygotes in 1,118 Adults and Children*

Haemoglobin Type	Children	Adults
SS	4	0
CC	7	3

Discussion

These results confirm the protective effect of haemoglobin-S among children with the sickle-cell trait and demonstrate a similar effect attributable to haemoglobin C among children with the haemoglobin-C trait. Mourant (1954) thought that the sickling gene might eventually be supplanted by a further mutation of the gene responsible for normal haemoglobin, giving rise to a haemoglobin both protective against *P. falciparum* malaria and relatively harmless in the homozygous state, and haemoglobin C would appear to fit this hypothesis. Terry *et al.* (1954) thought that the gene responsible for haemoglobin C synthesis occupied the same locus as those responsible for haemoglobins A and S. Further support for this theory came from the work of Hunt and Ingram (1958) and Ingram (1959), who found that the chemical differences between haemoglobins A, S, and C consisted in minimal changes in the fourth peptide of the beta chains, the glutamic acid residue of haemoglobin A being replaced by valine in haemoglobin S and lysine in haemoglobin C. It would thus seem that haemoglobin C is a mutation of either haemoglobin A or haemoglobin S, favourable in the heterozygous state in areas where *P. falciparum* malaria in endemic, and far less disadvantageous in the homozygous state than haemoglobin S. Although not reaching statistical significance in the present trial, the protective effect of haemoglobin C appears to be of a lower order than that of haemoglobin S. Allison (1955) calculated that in a population with a haemoglobin distribution similar to that of West Africa haemoglobin S would tend to be eliminated more rapidly than elsewhere because of the presence of haemoglobin C, and the latter would not need to be so advantageous in the heterozygous state as haemoglobin S for both genes to maintain their frequency.

As mentioned previously, Edington and Laing (1957) could not demonstrate the protective effect of haemoglobin C, and considered its existence to be most unlikely. They were able to show only an insignificant degree of protection for haemoglobin S, and it is possible that their results were marred by the fact that the majority of subjects in their survey were over the age of 11. During previous work in Ghana by one of these authors (Colbourne and Edington, 1956) it was concluded that sickling appeared to protect in the south but not in the north, possibly because of higher malarial pressure in the north, and it was stated that the important age-group in Accra, 6 months to 5 years, remained to be investigated. It would seem reasonable for haemoglobin C to be protective in all parts of Ghana, but, since acquired immunity is gained earlier in the north, to demonstrate the effect there would necessitate surveying a younger age-group than had sufficed in Accra.

The curious finding that adult carriers of the sickle-cell trait appear to get malaria more frequently than both the normal and carriers of haemoglobin C remains to be explained. Garlick (1960), in Nigeria, found the incidence of sicklers with blood-film-positive fevers and without signs of a non-malarial cause significantly lower

than the incidence of sicklers in two control groups. Wilson (1960) suggested that Garlick's findings of lower than normal parasite densities in sickling children at their first attendance could mean that they developed symptoms earlier and thus presented themselves at hospital sooner than those without haemoglobin S. Miller *et al.* (1956) advocated the mechanical protection theory of sickling consequent on malarial parasitization of erythrocytes containing haemoglobin S, and it is accepted that the pain crises of sickle-cell disease are caused by intravascular sickling in various parts of the body (Zuelzer, 1959). Therefore it is suggested that the lethal effects of intense parasitaemia by *P. falciparum* are prevented by sickling of parasitized cells, and that this protective effect is accompanied by early onset of symptoms. Thus adult policemen with the sickle-cell trait are unlikely to have contracted malaria more often than those without, but have developed symptoms with infections whose intensities were probably below the threshold required to produce symptoms in normal subjects. The incidence of malaria in policemen with the haemoglobin-C trait being lower than the normal suggests that the protective effect of haemoglobin C differs in its nature from that of haemoglobin S, and that it appears to remain demonstrable into adult life, possibly owing to an additive effect with the mechanism of acquired immunity.

Summary

Determination of the rate and intensity of *P. falciparum* infections among 840 Accra police children aged 1 to 6 years, correlated with the results of haemoglobin electrophoresis, confirmed the protective effect of haemoglobin S and demonstrated a similar effect for haemoglobin C in Ghana.

Among 278 adult policemen reporting sick with fever in Accra the incidence of positive blood films was highest in those with sickle-cell trait and lowest in those with the haemoglobin-C trait.

These findings support the theory that haemoglobin C is the result of a favourable mutation at the genetic locus responsible for haemoglobins A and S, and suggest that the protective effect exerted against malaria by haemoglobin S is due to sickling of parasitized erythrocytes and is accompanied by symptoms.

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REFERENCES

Allison, A. C. (1954). *Ann. hum. Genet.*, **19**, 39.
—— (1955). *Cold Spr. Harb. Symp. quant. Biol.*, **20**, 239.
—— (1957). *Exp. Parasit.*, **6**, 418.
Colbourne, M. J., and Edington, G. M. (1956). *Brit. med. J.*, **1**, 784.
Daland, G. A., and Castle, W. B. (1948). *J. Lab. clin. Med.*, **33**, 1082.
Edington, G. M. (1957). *J. clin. Path.*, **10**, 182.
—— (1959). In *Abnormal Haemoglobins*, edited by J. H. P. Jonxis and J. F. Delafresnaye, p. 290. Blackwell, Oxford.
—— and Laing, W. N. (1957). *Brit. med. J.*, **2**, 143.
—— and Lehmann, H. (1954). *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 332.
—— —— (1956). *Man*, **56**, 34.
Garlick, J. P. (1960). *Trans. roy. Soc. trop. Med. Hyg.*, **54**, 146.
Hunt, J. A., and Ingram, V. M. (1958). *Nature (Lond.)*, **181**, 1062.
Ingram, V. M. (1959). Ibid., **183**, 1795.
Itano, H. A., and Neel, J. V. (1950). *Proc. nat. Acad. Sci. (Wash.)*, **36**, 613.
Kaplan, E., Zuelzer, W. W., and Neel, J. V. (1953). *Blood*, **8**, 735.
Lehmann, H. (1956). *J. clin. Path.*, **9**, 180.
—— (1959). In *Abnormal Haemoglobins*, edited by J. H. P. Jonxis and J. F. Delafresnaye, p. 313. Blackwell, Oxford.
Lehmann, H., and Nwokolo, C. (1959). *Nature (Lond.)*, **183**, 1587.
—— and Raper, A. B. (1956). *Brit. med. J.*, **2**, 333.
McCormick, W. F. (1961). *Amer. J. med. Sci.*, **241**, 329.
Miller, M. J., Neel, J. V., and Livingstone, F. B. (1956). *Trans. roy. Soc. trop. Med. Hyg.*, **50**, 294.
Mourant, A. E. (1954). *Distribution of Human Blood Groups*, p. 184. Blackwell, Oxford.
Neel, J. V. (1956). *Ann. hum. Genet.*, **21**, 1.
Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C. (1949). *Science*, **110**, 543.
Raper, A. B. (1955). *Brit. med. J.*, **1**, 1186.
Rotter, R., Lutgens, W., Peterson, W., Stock, A., and Motulsky, A. (1956). *Ann. intern. Med.*, **44**, 257.
Smith, E. M., and Krevans, J. R. (1959). *Bull. Johns Hopk. Hosp.*, **104**, 17.
Terry, D. W., Motulsky, A. G., and Rath, C. E. (1954). *New Engl. J. Med.*, **251**, 365.
Walters, J. H., and Lehmann, H. (1956). *Trans. roy. Soc. trop. Med. Hyg.*, **50**, 204.
Wilson, T. (1960). Ibid., **54**, 283.
Zuelzer, W. W. (1959). In *Abnormal Haemoglobins*, edited by J. H. P. Jonxis and J. F. Delafresnaye, p. 105. Blackwell, Oxford.
—— Neel, J. V., and Robinson, A. R. (1956). *Progr. Hemat.*, **1**, 91

THE VIBRIOS OF THE RECENT CHOLERA-LIKE OUTBREAK IN HONG KONG

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Outbreaks of cholera were reported in 1961 from widely scattered Far Eastern countries, including Hong Kong, Macao, parts of the Chinese mainland, the Philippines, and Indonesia, after which an extensive campaign of anticholera inoculations was organized in active co-operation with W.H.O. (*J. Indian med. Ass.*, 1961).

We present in this paper the result of our bacteriological investigation of the causal organisms of these outbreaks, which we have identified as El Tor vibrios. This finding has important epidemiological implications because the cholera-like disease caused by El Tor vibrios, as distinct from the classical *Vibrio cholerae*, the causative organism of cholera, has not so far been known to occur outside a limited geographical area in the Indonesian archipelago. And since the El Tor vibrio is taxonomically quite distinct from *V. cholerae*, the protective value of the cholera vaccine (with *V. cholerae* antigens) against El Tor disease is open to doubt.

Attempts were made from this laboratory to collect vibrios isolated in these outbreaks for strain identification by phage-typing and examination of their possible relationship with the *V. cholerae* strains prevalent in the cholera endemic centres of India. A first instalment of six strains labelled as *V. cholerae* was obtained from Dr. D. J. M. MacKenzie, Director of Medical and Health Services, Hong Kong, through Dr. L. Mattsson, of the South East Asia Science Cooperation Office of Unesco. The same six strains were also forwarded by Lieutenant-Colonel O. Felsenfeld, of the S.E.A.T.O. Medical Research Laboratory, Bangkok, for phage-typing. All these strains were agglutinable, and a preliminary test for phage susceptibility indicated that they belonged to the El Tor type, a finding confirmed by the more detailed investigations reported below.

The results were communicated to Dr. MacKenzie with a request for the supply of all the remaining