their responses to types 2 and 3, after either two or three doses of vaccine. The difference in the type 1 responses after two doses, however, was much accentuated by giving a third dose. Though all infants in both groups responded, the geometric mean titre for the 6-months-old group was more than twelve times that of the 9-months-old group. These results suggest that the antigenic activity of the type 1 component of vaccine A which was given to the 6-months-old infants was greater than that of vaccine B given to the 9-months-old infants.

Conclusions

Placentally transmitted maternal antibody had fallen to non-inhibitory levels in almost all 6-months-old infants and in all 9-months-old infants tested.

The responses of infants in these age groups to two doses of vaccine were good to the type 2 and 3 components, all infants responding, but those to type 1 were not quite so satisfactory because 3 out of 15 6-months-old and 6 out of 14 9-months-old infants failed to show a rise in antibody level. The responses after three doses, however, were much more satisfactory, all infants responding to type 1 giving higher antibody levels, and the responses to types 2 and 3 being very much higher than after two doses.

The 6-months-old infants responded better to type 1 than the 9-months-old infants, and this difference was most pronounced after the third dose. This was probably due to the difference in the antigenic activity of type 1 in the two vaccines used. Though a small difference was observed when measured in monkeys, it was not as marked as in the infants. It is important, therefore, that only vaccines highly potent in the type 1 component should be used for immunizing infants, and that three doses given at monthly intervals should constitute the primary course at this age.

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Since 1955 the medical and health services in Mauritius have been considerably expanded under a plan for capital expenditure. Part of the service was concerned with an island-wide campaign to eliminate malaria, which was previously regarded as a scourge of the colony. The effectiveness of the campaign can be gauged by a comparison of cases within a period of the last 12 years: in 1945 more than 3,000 cases of malaria were treated in hospitals, with a total of 148 deaths; in 1957 only four cases were treated in hospitals, with no deaths reported.

TESTS FOR PHENYLKETONURIA

RESULTS OF A ONE-YEAR PROGRAMME FOR ITS DETECTION IN INFANCY AND AMONG MENTAL DEFECTIVES

BY

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The diagnosis of phenylketonuria is important for three different reasons: in young infants the detection of this metabolic error permits treatment with a low-phenylalanine diet in order to prevent or minimize mental deterioration; the ascertainment of all cases of phenylketonuria among mental defectives would permit an accurate estimate of the frequency in the population of the gene responsible; to the paediatrician the differentiation of phenylketonuria from other forms of mental deficiency is always important, not least because of the light it throws on the chances of later children in the family being affected. For these reasons any test which makes the diagnosis of phenylketonuria simpler or more reliable is to be welcomed.

Several workers have reported that phenylketonurics treated with a low-phenylalanine diet from the age of a few weeks developed normal intelligence, while in cases where treatment was delayed the I.Q. usually remained below the average, and in untreated phenylketonurics the modal I.Q. was 10 (Horner and Streamer, 1956, 1959; Armstrong, Low, and Bosma, 1957; Woolf, Griffiths, Moncrieff, Coates, and Dillistone, 1958; Hsia, Knox, Quinn, and Paine, 1958; Brimblecombe, Stoneman, and Maliphant, 1959; Armstrong, Centerwall, Horner, Low, and Weil, 1959). This emphasizes the importance of early detection if irreversible mental deterioration is to be avoided. Programmes aimed at detecting all cases in a community at a very early age have been suggested or initiated (Woolf, Griffiths, and Moncrieff, 1955; Centerwall, 1957a, 1957b; Hsia, 1957; Woolf *et al.*, 1958; Baird, 1958). We report here the findings of such a detection programme carried out in Cardiff over the period March 1, 1958, to March 1, 1959, and also a survey of four institutions for mental defectives. "Phenistix," a strip which is dipped into the urine like litmus paper, has been found to be a simple and satisfactory test for phenylketonuria.

Method

The mother of every child born in Cardiff was asked to bring a fresh specimen of her baby's urine to the infant-welfare clinic when the baby was 3 weeks old or as soon as possible after. A bottle containing a little chlorbutol as a preservative was provided by the health visitor. Ferric chloride solution (5%) was added drop by drop to the urine until a definite colour appeared.

Results

Though there were 4,530 live births in Cardiff during the year, only 1,192 specimens of urine were collected and tested. The mothers of the 73.7% of babies that were not tested generally complained that it was

impossible to get a specimen of urine, the baby passing urine only when wearing a napkin.

Of the specimens tested, 51 were strongly alkaline, and were rejected. Out of the remaining 1,141 specimens, one gave a positive reaction for phenylpyruvic acid. The infant was admitted to Llandough Hospital under Professor A. G. Watkins, and phenylketonuria was confirmed by chromatography of the serum for phenylalanine. Treatment on a low-phenylalanine diet was started at the age of 1 month. On her first birthday this child had a G.Q. of 94 (Griffiths scale), and her weight was 24 lb. 13 oz. (11.25 kg.).

Seven other infants passed urine that gave a green colour with ferric chloride, exactly like that given by phenylpyruvic acid. However, laboratory investigations by Mr. R. A. Saunders showed that the condition was not phenylketonuria. The positive reactions were found to be due to *p*-hydroxyphenylpyruvic acid. These findings will be described in greater detail elsewhere.

Since December, 1958, infants' urines that were being tested with ferric chloride have also been tested with phenistix. The phenistix method was found to be so much simpler and the results so concordant (except when the urine contained *p*-hydroxyphenylpyruvic acid, see below) that it is proposed to extend the use of phenistix to the infant-welfare clinics when a supply is available, and, perhaps, later to issue the strips to health visitors for use in the home.

Mental-deficiency Survey

In addition to the detection programme for infants, all the children attending a junior school for educationally subnormal children and two centres for mentally defective children, as well as children in a local institution for mental defectives, had their urines examined, using the ferric chloride test. Out of 279 children, two cases of phenylketonuria were found. One of these was a 7-year-old boy who is now being treated on a diet low in phenylalanine; he is much easier to manage than before treatment. The other case detected was that of a 13-year-old who was regarded as untreatable.

Phenistix strips have also been used for examining a urine specimen from one of these children. The reagent strips were found far more convenient to use than the ferric chloride solution, and the results were in complete agreement.

Discussion

Detection of phenylketonuria must be made as early as possible if the affected infant is to have a reasonable chance of growing up mentally normal. These infants do not excrete phenylpyruvic acid at birth, and the best age for testing for it is not easy to determine: the first appearance of phenylpyruvic acid in the urine has been reported at ages ranging from 6 days to 6 weeks. About half the children in Great Britain are born in a hospital or nursing-home, where the organization of tests would be easy, but the infants leave after 10 days, when a large proportion of phenylketonurics do not yet excrete phenylpyruvic acid; there is a serious risk that the child would not be retested if it were known that his urine had already been examined, and this risk becomes greater as the difficulty of testing increases. Three weeks has been suggested as a compromise age for testing the urine—an age when the great majority of phenylketonurics will be excreting phenylpyruvic acid, but mental deterioration will still be slight (Woolf *et al.*, 1958). Retesting at about two months is recommended. Armstrong *et al.* (1959) have reported on six phenylketonurics observed from earliest infancy; some excreted large amounts of phenylpyruvic acid by the seventh day of life, while one did not excrete this acid until 5 weeks old. They suggest that if the urine were tested at the age of 2 weeks most cases would be detected.

The difficulty in obtaining a specimen of urine was by far the greatest single factor limiting the use of the test. Of the children born in Cardiff during the year, only a quarter had their urine tested. If a wet napkin could be tested for phenylpyruvic acid, nearly every child could be tested by the health visitor either at home or at the infant-welfare centre. Phenistix strips seem, at the moment, the best way of using a wet napkin, but there is room for improvement, particularly when the napkin is merely moist.

Where an appreciable volume of urine is available there is a choice of tests. Ferric chloride solution is the most widely used reagent; it should be used without mineral acid. The addition of quite a small amount of sulphuric or hydrochloric acid can invalidate the ferric chloride reaction by depressing the *p*H below that at which any appreciable colour is produced (Woolf, in preparation; cf. Følling and Sydnes, 1958).

p-Hydroxyphenylpyruvic acid occurs in the urine of infants fed large doses of tyrosine or phenylalanine (Levine, Marples, and Gordon, 1939, 1941; Levine, Dann, and Marples, 1943; Woolf and Edmunds, 1950), of sufferers from scurvy (Rogers and Gardner, 1949; Morris, Harpur, and Goldbloom, 1950), of adults and children with hepatic cirrhosis (Felix, Leonhardi, and Glasenapp, 1951; Woolf, unpublished), and, as briefly reported above, of a number of normal infants (Gibbs and Woolf, in preparation). It is difficult to distinguish *p*-hydroxyphenylpyruvic acid from phenylpyruvic acid by using ferric chloride or 2:4-dinitrophenylhydrazine; the two acids can be differentiated by using phenistix and observing the colour on the strip, but not if the reagents on the strip are leached out into the urine. The use of Briggs's reagent (cf. Woolf and Edmunds, 1950; Berry and Woolf, 1952) allows these two acids to be distinguished.

A positive reaction by any test must not be taken as conclusive proof of the diagnosis unless supported by, for example, serum phenylalanine determination. In the case of a "high-risk" group, such as the sibs of known cases of phenylketonuria, it is in any case advisable to carry out this laborious procedure at the ages of, say, 1, 2, 3, and 6 weeks, as well as using one of the simple urine tests as often as possible. Armstrong *et al.* (1959) suggest the determination of serum phenylalanine after one or two days of life in such cases.

The results of a detection programme in infants such as that reported here are of little use for determining the frequency of the gene in the population, because the high "failure rate"—75%—in this case is almost certainly not random. The great majority of phenylketonurics are eventually placed in institutions, and by determining the number of phenylketonurics in mental-deficiency institutions in an area the incidence in the population can be ascertained with fair accuracy. A large number of urine specimens have to be tested, and the test used should be as simple and speedy as possible. Dipping a

phenistix strip into the specimen is the quickest test available, and requires least in the way of apparatus, chemicals, or special skills.

Summary

A programme aimed at testing for phenylketonuria the urine of all infants born in Cardiff achieved only a 25% success. None the less, one case was detected and treated successfully. It is difficult to get a suitable specimen of urine, and a simpler test that can use a wet napkin is necessary.

The complete ascertainment of the frequency of phenylketonuria in the population requires the testing of all mental defectives. A simple and speedy test is very desirable.

If the ferric chloride test is used the urine should not be acidified.

A new test strip, phenistix, is a simpler and quicker method of testing urine for phenylpyruvic acid than methods hitherto used. It may also provide a partial answer to the problem of testing on the napkin. Unlike ferric chloride and 2:4-dinitrophenylhydrazine, it does not give a false-positive reaction with *p*-hydroxyphenylpyruvic acid, which has been found in the urine of a number of infants in the Cardiff survey.

We thank Sir George Pickering for providing laboratory facilities while the Population Genetics Research Unit's laboratories were being built; Professor A. G. Watkins for permission to refer to the phenylketonuric infant treated by him; Mr. R. A. Saunders for permission to quote his laboratory findings; Mrs. Philips, S.R.N., mental-health worker, Cardiff City Council, for her untiring assistance in collecting and testing the specimens of urine; and Ames and Co. for samples of "phenistix."

APPENDIX

Ames and Co. have produced "phenistix," a test strip which can be dipped into a specimen of urine and which indicates the presence of phenylpyruvic acid by turning green. Phenistix consists of a strip of thick absorbent paper, 6 by 0.4 cm., one end of which is impregnated with a ferric salt, a magnesium salt, and a buffer (cyclohexylsulphaminic acid). The stick can be compared with a colour chart on the bottle, giving the phenylpyruvic acid content of the urine. The usefulness of phenistix was investigated (cf. Rupe and Free, 1958; Baird, 1958).

The *p*H of a solution made by soaking phenistix in water was found to be 2.3, very close to the optimum *p*H for the reaction between ferric chloride and phenylpyruvic acid (cf. Berry and Woolf, 1952).

A specimen of urine from a child with phenylketonuria was used in preference to a synthetic standard. The specimen has been found, by the method of Berry and Woolf (1952), to contain 106 mg. of phenylpyruvic acid per 100 ml. Two dilution series were prepared: (i) with normal urine of about the same specific gravity, and (ii) with distilled water. Since the ratio of phenylpyruvic acid to total nitrogen is fairly constant, but urine from young infants is often dilute, (ii) probably represents the more important case. Each diluted specimen was tested with phenistix, with ferric chloride, and with 2:4-dinitrophenylhydrazine in 2N hydrochloric acid (saturated solution).

It was found that the phenistix strip had to be quickly dipped in and out of the urine; the reagents were leached out if the strip was left in the urine for much longer than a second or two. Maximum colour development occurred after one minute at a room temperature of about 20° C.—not after half a minute, the time recommended by the manufacturers for reading the strips. Unlike the ferric chloride test, the time to maximum colour development was independent of the temperature of the urine. For series (i), for a given concentration of phenylpyruvic acid, the colour on the strip corresponded closely to the colour on the chart; for series (ii) the strip was appreciably more bluish than the chart colour, though not so much so as to lead to uncertainty about the presence or absence of phenylpyruvic acid. The limit of detectability with phenistix for series (i) was 10 mg. per 100 ml., and for series (ii) it was 5 to 10 mg. per 100 ml.; with the ferric chloride test the limit of detectability was 5 mg. per 100 ml. for both series (i) and (ii); with 2:4-dinitrophenylhydrazine reagent the limit of detectability was 10 mg. of phenylpyruvic acid per 100 ml. of urine or aqueous solution. Thus all three tests have virtually the same sensitivity; urine from a phenylketonuric almost always has far more than 10 mg. of phenylpyruvic acid per 100 ml., about 100 mg. per 100 ml. being a usual value for children.

Reactions of Ferric Chloride and of Phenistix With Various Substances Added to Normal Urine

Substance	Phenistix	Ferric Chloride Solution
Phenylpyruvic acid ..	Blue-green, maximal in one minute, fading slowly	Blue-green. Eventually fades to yellow
p-Hydroxyphenylpyruvic acid	Ephemeral green, fading in 2–3 seconds	Blue-green.* Yellow with excess ferric chloride
Pyruvic acid	Golden yellow	Deep golden yellow
o-Hydroxyphenylpyruvic acid	Green*, maximal in about one minute	Initial red-brown turns green or blue*. Eventually fades to mauve
α-Ketobutyric acid ..	Faint brown-purple	Intense purple fading to red-brown in 1–2 seconds
"Maple syrup urine"†	Nil	Grey, with a greenish tinge
Sodium salicylate ..	Stable intense mauve	Stable intense mauve
Homogentisic acid ..	Nil (brown with stronger solution)	Fleeting green. Brown with stronger solution
o-Hydroxyphenylacetic acid	Very pale mauve	Mauve
3-Hydroxyanthranilic acid	Immediate yellow turns green* over one minute. Later brown	Immediate deep brown
Xanthurenic acid ..	Nil	Deep green*. Later brown
Vanillic acid	Brown	Red-mauve turning deep brown
Phenol	Nil	Deep blue-violet
Lysol	,,	Green*
"Dettol"	,,	Yellow

* Reaction difficult to distinguish from that of phenylpyruvic acid.
† Urine from a case of "maple syrup urine disease" (Mackenzie and Woolf, 1959).

A number of substances that might occur in urine, as rare endogenous products, or because of administration of drugs, or as contaminants, were examined, in solution in normal urine, for their ability to interfere with the ferric chloride and phenistix reactions. The results are shown in the Table.

Pieces of Turkish towelling, as used for babies' napkins, were wetted with urine from a phenylketonuric and a phenistix strip was applied. When the cloth was thoroughly wet, but not so wet that any urine could be squeezed or wrung out of it, a positive result was obtained on the strip, but only if the strip was placed between two layers of the wettest part of the cloth and firm pressure applied for several seconds. If the napkin was merely moist to the touch, as happens after a wet napkin is removed and allowed to stand for some hours, the strip was not moistened and no positive result was obtained. In this case a positive result could still be obtained as follows: the strip was dipped into water for the briefest possible moment and the wet end squeezed hard between the wettest parts of the napkin for several seconds; the buffered ferric salt dissolved and printed on to the napkin, where, if positive, a green patch appeared, the size and shape of the end of the strip. This technique was rather less sensitive to low concentrations of phenylpyruvic acid than the conventional one, and, owing to the different ratio of ferric salt and urinary substances, less specific, giving a green colour with *p*-hydroxyphenylpyruvic acid. It is a convenient way of applying buffered ferric salt to the napkin, and avoids the spreading effect of dropping ferric chloride solution on to it.

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ORAL TREATMENT OF PERNICIOUS ANAEMIA WITH VITAMIN B_{12} AND DESICCATED HOG DUODENAL EXTRACT

BY

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Oral administration of a substance with intrinsic factor activity is a rational approach to the treatment of pernicious anaemia. However, treatment by adequate parenteral vitamin B_{12} is so consistently effective in maintaining a normal state of the blood and bone marrow, and in safeguarding against the development of neurological complications, that any other form of therapy must be shown to be as effective before it can supplant injections of vitamin B_{12} in the routine management of the disease. Oral therapy has an obvious attraction, and Wilkinson (1949) has shown that excellent results may be obtained by the use of a relatively crude preparation of desiccated hog's stomach. In recent years preparations have been used in the treatment of pernicious anaemia containing, in tablet form, a small amount (5–15 μg.) of vitamin B_{12} and an extract of desiccated hog duodenal or pyloric mucosa. Evidence has been accumulating that this form of therapy usually results in a good initial response, but with continued treatment haematological remission and normal levels of vitamin B_{12} in the serum are not maintained (Bastrup-Madsen, 1957; Kristensen *et al.*, 1957; Lowenstein *et al.*, 1957; Killander, 1958). It has been shown that many patients treated with such oral preparations ultimately have reduced intestinal absorption of vitamin B_{12} when hog pyloric mucosa is supplied as intrinsic factor (Lowenstein *et al.*, 1957; Schwartz *et al.*, 1957; Stokes and Pitney, 1958). This leads ultimately to deficiency of vitamin B_{12} and clinical relapse.

The present report describes the results of treatment of pernicious anaemia patients with "biopar" tablets over a period of five years, and is an extension of a previous report on treatment with biopar for one year (Blackburn *et al.*, 1955). The changes in the peripheral blood, the bone marrow, and the levels of vitamin B_{12} in the serum have been followed during this period of treatment. In addition, tests were made for skin sensitivity to hog intrinsic factor prepared by the same method as used in biopar tablets.

Patients Studied and Details of Treatment

The results of oral treatment with tablets containing crystalline vitamin B_{12} and a desiccated extract of hog duodenal mucosa (biopar and biopar forte) have been studied in 22 patients with Addisonian pernicious anaemia. In all cases the diagnostic criteria included megalocytic anaemia, megaloblastic erythropoiesis in the bone marrow, histamine-fast gastric achlorhydria, and the absence of evidence of steatorrhoea or other recognized cause of megaloblastic anaemia. Each tablet of biopar contained 6 μg. of vitamin B_{12} and 30 mg. of the intrinsic factor preparation; each tablet of biopar forte contained 15 μg. of vitamin B_{12} and 35 mg. of the intrinsic factor preparation.

Thirteen patients (Cases 1–13) who had previously been treated with parenteral vitamin B_{12}, and in whom steady and normal levels of haemoglobin and red-cell count had been maintained during at least one year, were transferred to treatment with biopar in November, 1953. From then until June, 1955, these patients received one tablet of biopar daily. The dose was then increased to two tablets daily until October, 1956. In 1956 the production of biopar tablets was stopped, and they were replaced by biopar forte. From October, 1956, until March, 1959, treatment was given with biopar forte, two tablets daily.

Nine patients with previously untreated pernicious anaemia who had shown a satisfactory initial response to oral treatment were included in the study. Four of these patients (Cases 14–17) were given initially five tablets of biopar daily. This was continued for two to nine months, until June, 1955, when they were transferred to the above scheme of dosage. Five patients (Cases 18–22) received biopar forte, five tablets daily for six months, and then continued treatment with two tablets daily.

The results during the first year of treatment with biopar in 10 of these patients have already been published (Blackburn *et al.*, 1955). Of the 22 patients included in the present study, five did not complete the trial for reasons detailed below.

Methods of Investigation

In all cases determinations of haemoglobin level (M.R.C. grey wedge photometer; oxyhaemoglobin—