THE CONTROL OF BACILLARY DYSENTERY

BY

R. W. FAIRBROTHER, M.D., D.Sc., F.R.C.P.

Lieut.-Col., R.A.M.C.

Bacillary dysentery is an important endemic disease of this country, and is characterized by frequent localized outbreaks which are usually unrelated in origin and are caused almost exclusively by the Sonne and Flexner organisms. Its incidence has increased considerably since the outbreak of the war, but this is not unexpected, as under wartime conditions it is often necessary to accept relatively low standards of hygiene; this applies particularly to troops under active-service conditions.

The failure to control bacillary dysentery in this and other countries has not been satisfactorily explained, but, following recent developments in laboratory technique, evidence is now accumulating which may provide a solution to this problem. The isolation of *Bact. dysenteriae* has been greatly facilitated, and as a result it has been possible to establish several facts which indicate that the procedures at present adopted to control the disease require careful and critical examination. Knowledge of the epidemiology of dysentery was previously derived from media with little, if any, selective action for *Bact. dysenteriae*, and was consequently often inaccurate, owing to the frequent failures to isolate the causative organisms not only from convalescents but also from actual cases of the disease. By the use of the highly selective desoxycholate-citrate medium the following points have been firmly established by many independent workers: symptomless carriers are common during an outbreak of dysentery; convalescent and symptomless carriers may excrete intermittently the organisms for long periods; sulphonamide therapy has proved satisfactory in Flexner infections, but not particularly successful in clearing Sonne infections (*E.P.H.L.S. Bull.*, 1942, 1943; Watt, Hardy, and De Capito, 1942; Fairbrother, 1943; Hardy and Watt, 1944).

The epidemiology of bacillary dysentery is thus a complex problem, but considerable progress has been made during the past two years. It is now clear that rigorous control measures and a long-term policy are necessary if the disease is to be eradicated from this country. In dealing with outbreaks it is essential that a thorough bacteriological investigation should be carried out on all clinical cases and close contacts, particularly persons concerned with the handling of food, and that all infected persons should be treated and, if possible, segregated until free from infection.

Several important questions, however, remain unanswered: one is the number and frequency of negative faecal examinations required before an individual can be declared free from infection; the second is the treatment of Sonne dysentery. The results given below confirm previous observations about the persistent but intermittent excretion of *Bact. dysenteriae* and provide further data for consideration in discussing these problems.

Technique

Samples of faeces, usually received within one or two hours of collection, were thoroughly suspended by means of sterile glass rods in small amounts of physiological saline. The thick suspensions were then plated on to desoxycholate-citrate (D-C) plates: in the case of fluid faeces, cultures were prepared directly with flakes of mucus. After overnight incubation at

37° C. suspicious colonies were selected for biochemical and serological tests; all plates were then reincubated for a further 24 hours, after which, if suspicious colonies had not been found, a negative result was reported.

The results of this investigation will be considered in two sections: (1) the carrier rate in bacillary dysentery; (2) the treatment of Sonne dysentery.

1. The Carrier Rate in Bacillary Dysentery

A favourable opportunity of studying the incidence of dysentery carriers was afforded by the arrival in this country of Italian P.O.W. from the Middle East campaign. In order to provide some sort of hygienic control the faeces of all giving a history of dysentery or enteric fever during the past three or four years were subjected to bacteriological investigation. Many samples of faeces were consequently examined, and, rather contrary to accepted opinion, it was found that the carrier state was not infrequent. From some 2,500 individuals *Bact. dysenteriae* was isolated on 245 occasions—i.e., the carrier state existed in approximately 10% of those with a recent history of bacillary dysentery. Even this figure must be considered a low estimate, since in many instances it was only possible to examine one sample of faeces from each man; as it is well established that the excretion of *Bact. dysenteriae* by carriers is intermittent, some carriers were almost certainly missed because of the limited scope of the examinations. The types of *Bact. dysenteriae* isolated from the 245 carriers are given in Table I.

TABLE I.—Types of *Bact. dysenteriae* isolated from 245 Italian Carriers

Flexner	170	Schmitz	11
Shiga	57	Sonne	7

The majority of these carriers did not show any dysenteric symptoms; looseness of the bowels was uncommon, while the presence of blood and mucus was exceptional. There was thus no clinical indication of the infection in most of the carriers. Cultures prepared on MacConkey plates from these cases were almost invariably negative.

All carriers were given a routine course of sulphaguanidine—6 g. three times on the first day, and then 3 g. three times a day for the next five days, making a total dosage of 63 g. in six days. In order to ensure the complete elimination of the drug clearance tests were not started until five days after completion of the course, and consisted originally of three successive negative examinations of the faeces (*A.M.D. Bull.*, 1942; *E.P.H.L.S. Bull.*, 1943). During later stages of this investigation abundant evidence was, however, forthcoming that 3 successive negative results did not constitute a reliable criterion of freedom from infection: *Bact. flexneri* was isolated on one occasion after 8 successive negative results, *Bact. shigae* after 6 successive negatives, while *Bact. sonnei* was frequently isolated after 3 and on one occasion even after 10 previous negative results. In consequence, more rigorous tests consisting of 12 successive negative results were introduced to determine clearance of the infection. It follows from these observations that the earlier results of sulphaguanidine therapy in this series were not adequately controlled. However, according to the different standards used, the treatment appeared to

be successful in 227 cases; the 16 failures were—Flexner 12, Shiga 2, and Sonne 2. The majority of the 16 cases were cleared by a second course of sulphaguanidine.

These results indicate that the carrier state in bacillary dysentery is relatively common and persistent, as in many cases the acute stage of the disease had occurred some two or three years previously. The carrier state does not usually give rise to symptoms, and therefore clinical evidence of its existence is not present. The results also suggest that sulphaguanidine is a satisfactory agent for the treatment of dysentery carriers; it is important, however, to note that only a few Sonne carriers were found. In view of the inadequate clearance tests used for most of the cases, it is impossible to give a definite opinion on this matter. While it is generally agreed that Flexner infections respond well, clinically and bacteriologically, to sulphaguanidine therapy, some of these observations have been based on unsatisfactory clearance tests, which were either too few in number or were carried out immediately after completion of the sulphaguanidine treatment. There is, however, much evidence to support this contention, which the above results tend to confirm.

2. Treatment of Sonne Dysentery

Sonne dysentery, particularly in adults, is the mildest form of the disease. The usual course is merely a transient looseness of the bowels lasting 24 to 48 hours, while in most outbreaks symptomless carriers are found during routine investigations. Nevertheless, in spite of the trivial clinical signs, *Bact. sonnei* tends to persist in the faeces for relatively long periods after clinical recovery, and has frequently proved resistant to treatment with the sulphonamides. Sulphaguanidine has been disappointing in producing bacteriological cure, and a new derivative, succinyl-sulphathiazole, is now being tried (M.R.C. War Memorandum, 1943). A small supply of this drug became available, and its value in Sonne dysentery has been investigated.

Of the 92 cases of Sonne dysentery studied 73 were in members of the A.T.S. A few of these were sporadic cases, but the majority were involved in small, localized outbreaks of some 8 to 12 cases, and were either of a mild clinical type or symptomless carriers. In each outbreak there were also a few cases showing slight transient diarrhoea, but from these *Bact. sonnei* was never isolated, in spite of repeated tests. While these cases were probable mild Sonne infections they have not been included in this investigation.

In view of the trivial form of the disease and the small supply of succinyl-sulphathiazole available, sulphonamide therapy was withheld from the majority until it appeared that the carrier state was developing. The patients were placed on a routine form of therapy consisting of rest in bed, saline purges, and light diet; bacteriological examinations of the faeces were carried out immediately, and then at frequent intervals until a positive result was obtained, after which tests were made at intervals of two or three days. As soon as a negative result was reported daily tests were made until 12 successive negative results had been given; during this period a few doses of a saline purge were administered. On an average some 18 tests were carried out on each patient. Sulphonamides were given only when the excretion of *Bact. sonnei* had persisted for three or four weeks after the onset of the disease. In these cases clearance tests were not started until at least five days after cessation of the sulphonamide, in order to ensure that the drug had been completely eliminated. This interval is an important factor in obtaining accurate results from the clearance tests. It was found that while negative results were almost invariably given immediately after the treatment, cases not infrequently became positive six or seven days later; only a few colonies appeared at first in positive cultures, but later a profuse growth of *Bact. sonnei* tended to develop. Failure to allow adequate time for the elimination of the sulphonamide from the faeces is thus an important source of error in carrying out clearance tests.

The various groups of cases are not strictly comparable because of the late stage of the disease at which the sulphonamides were usually given; it is therefore proposed to consider them separately according to the type of treatment received: (a) routine with sulphonamides; (b) sulphaguanidine; (c) succinyl-sulphathiazole.

(a) *Cases on Routine Therapy Without Sulphonamides.*—76 mild or asymptomatic cases of Sonne dysentery received the routine course of therapy—i.e., rest in bed, saline purges, and light diet. All recovered clinically within 48 to 72 hours. The prescribed tests for bacteriological cure (12 successive negative results) were carried out in all except a few of the earlier cases, in which the number of clearance tests was that generally recognized—namely, three. Of the 76 cases 45 (59%) resolved completely, as indicated in Table II.

TABLE II.—Clearance of Infection in Sonne Dysentery (76 Cases) (without Sulphonamide Therapy)

Period of Disease	Number Cleared	Period of Disease	Number Cleared
1st week	24	3rd week	4
2nd "	15	4th "	2

24 cases (32%) cleared spontaneously during the first week of the disease and a further 15 in the second week. A total of 39 (52%) cases thus resolved completely within two weeks of onset. A few cleared in the third and fourth weeks, but during this period treatment with sulphonamides was generally instituted, and it is highly probable that if left untreated more cases might have cleared spontaneously. Therefore in evaluating the effects of sulphonamides in mild cases of Sonne dysentery it is important to appreciate that in a high proportion of cases the carrier state is only of a transient nature and disappears early in convalescence; in this investigation at least 60% of the cases became bacteriologically clear within three weeks on ordinary routine treatment. When the infection persisted the excretion of *Bact. sonnei* was often intermittent: on one occasion a positive result was obtained after 10 successive negative examinations.

(b) *Treatment with Sulphaguanidine.*—Altogether 22 cases were treated with sulphaguanidine. The routine course (see above) was originally given, but, as some cases failed to respond satisfactorily, treatment was later extended to 8 or 9 days, making a total dosage of 81 or 90 g. Eight cases received the drug during the initial active stage of the disease; in two instances the symptoms were moderately severe, pyrexia was present, and blood and mucus were observed in the stools. Clinical response to the sulphaguanidine was invariably good, but from the bacteriological aspect the results were disappointing. Satisfactory clearance tests were given by 5 out of the 8 cases (63%). Of 13 convalescent carriers who also received a course of sulphaguanidine the infection was cleared in 11. One persistent carrier, however, had several courses of this drug, including one of 200 g., without success. A few cases received the sulphaguanidine in the form of an enema—10 g. in 4 oz. of water twice a day for 5 days. The response was similar to that following oral administration of the drug: a few convalescent carriers were cleared, but there was no effect on two persistent carriers. The use of this route for administering the drug was consequently abandoned, as there were no apparent advantages over the oral method.

(c) *Treatment with Succinyl-sulphathiazole.*—14 convalescent carriers, 1 relapsed case, and 2 persistent carriers were given the following course of succinyl-sulphathiazole: 6 g. three times a day on the first day and 3 g. three times a day for 7 or 8 days, making a total dosage of 81 or 90 g. The results were frankly disappointing, being little better than those of sulphaguanidine therapy. Satisfactory clearance tests were given by 13 out of 14 convalescent carriers, but positive cultures were obtained after treatment from the relapsed case, both persistent carriers, and one of the convalescent carriers.

These results indicate that sulphaguanidine and succinyl-sulphathiazole have only a limited effect in Sonne dysentery. While the clinical response is good, their value in clearing *Bact. sonnei* from the faeces is less satisfactory: two persistent carriers have continued to excrete *Bact. sonnei* for some nine months in spite of repeated courses of both drugs. A further case of a moderately severe type continued to excrete the organisms although treated with both drugs, but eventually cleared spontaneously two weeks after the faeces had been persistently and strongly positive in spite of recent treatment with succinyl-sulphathiazole and, earlier, with sulphaguanidine; altogether this case gave 10 positive and 26 negative results over a period of 16 weeks.

In view of the mild nature of Sonne infections, sulphonamide therapy is usually required to effect a bacteriological rather than a clinical cure. While these drugs are useful for this purpose, they have not proved entirely successful, and it is hoped that a more effective compound will eventually be produced.

There is no satisfactory explanation for the not infrequent failure of the sulphonamides to effect a bacteriological cure in Sonne infections. The *in vitro* activity of the sulphonamides on various strains of *Bact. dysenteriae* was investigated, using the ditch-plate technique, as it was possible that the irregular results might have been due to variations in strain resistance.

A solution to the problem was not, however, forthcoming. Flexner and Sonne strains behaved in a comparable manner throughout; succinyl-sulphathiazole exercised a definite inhibitory activity on both organisms, but sulphaguanidine proved relatively ineffective; while evidence was not obtained that the persistence of infection was due to the development of sulphonamide-resistant strains of *Bact. sonnei* (Beemer and Fairbrother, 1944). A probable explanation of the uncertain action of the sulphonamides in Sonne infections is that, as Sonne dysentery is usually a mild disease, the organisms multiply on, rather than in, the mucous membrane and do not produce the severe ulceration which is usual in the other types of bacillary dysentery. The Sonne organisms thus tend to lead a more saprophytic existence than the other members of the dysentery group; this biological variation is probably reflected in the biochemical activity of *Bact. sonnei*, which is the only pathogenic member of the genus *Bacterium* to ferment lactose. In consequence of its relatively saprophytic existence, contact with the organisms by the drug is more difficult to effect than in the case of the more parasitic members, which multiply in the damaged mucosa. During sulphonamide therapy most of the organisms are eliminated, but a few, perhaps protected by faeces, escape the action of the drug and propagate when the inhibitory action of the sulphonamide is removed. There appears to be an analogy between Sonne dysentery and streptococcal tonsillitis: in both conditions the clinical response to sulphonamide therapy is often good, while the bacteriological response is poor. In neither case is the failure to effect a bacteriological cure essentially due to the development of drug resistance by the organisms.

Discussion

These results confirm previous observations and indicate that in order to control bacillary dysentery it is essential that (1) all outbreaks of the disease must be subjected to thorough bacteriological investigations, and (2) stringent clearance tests must be satisfied before a case can be considered free from infection.

The eradication of the disease from this country requires the detection and treatment of all carriers of *Bact. dysenteriae*. The important part played by the carrier in the persistence and spread of bacillary dysentery in the community is now firmly established, and is well illustrated in the epidemic reported by Hailwood (1944). In this outbreak 292 cases of diarrhoea occurred in a unit of some 900 men while at a practice camp. The faeces from only 81 cases were positive for *Bact. sonnei*, but bacteriological investigations were not started until some 14 days after the onset of the outbreak, by which time the infection would have cleared spontaneously in many cases. The source of the infection was proved to be a chronic symptomless carrier who had had no symptoms of dysentery since a moderately severe attack some 12 months previously, but whose stools still yielded a heavy growth of *Bact. sonnei*. This man had been mainly employed in cutting bread-and-butter.

The detection of carriers is unfortunately a matter of considerable difficulty, as many exist and some have never shown the symptoms of the disease and can only be discovered by laboratory methods, which should consequently be widely applied as soon as an outbreak is reported. The introduction of highly selective media, such as desoxycholate citrate, has greatly simplified the isolation of *Bact. dysenteriae* and has revealed an unexpectedly high incidence of carriers. Results obtained by the use of old media, such as MacConkey's, are completely valueless in dealing with convalescent or symptomless carriers, and it is doubtful if these media fulfil any useful function in the examination of faeces.

The high incidence of carriers, particularly of Flexner strains, found in Italian soldiers indicates that the development of the carrier state in bacillary dysentery is a common feature of the disease. This raises a hygiene problem of great importance. Bacillary dysentery has been widespread among our troops in N. Africa, and in some 70% of cases in which bacteriological investigations were carried out (incidentally only a small percentage of the total number) *Bact. flexneri* was the responsible organism. Sulphaguanidine therapy was not universally applied, little or no attempt was made to discover symptomless carriers, and in the majority of cases bacteriological control in the form of clearance tests was not possible. There is con-

sequently little doubt that a number of carriers will have developed and that such individuals will be a potential source of infection on return to Great Britain. This has already proved to be the case. *Bact. dysenteriae* has been isolated not infrequently from men returning and giving a history of dysentery some time previously but not at the time showing actual symptoms of the disease. A routine check of all persons returning from abroad is not practicable. It is therefore necessary that if such individuals are involved in any outbreak they should be subjected to rigorous tests before being considered free from infection.

Another essential factor in the control of dysentery is the establishment of a satisfactory standard for the clearance tests. It is obvious that the criterion of freedom from infection at present generally accepted—i.e., three successive negative results—is quite unreliable. During this investigation positive results have been obtained in Shiga, Flexner, and Sonne infections after as many as 6, 8, and 10 successive negatives respectively, while positive results after 3 successive negatives have been encountered with great frequency in Sonne infections. Stringent measures must therefore be adopted in order to provide a satisfactory criterion of bacteriological cure, and it is recommended that (i) where laboratory facilities are readily available at least 12 successive daily negative results should be obtained, and (ii) where laboratory facilities are poor 12 successive negatives should be obtained over a period of at least 3 weeks; in all cases tests should not be started until 6 days after cessation of treatment if sulphonamides have been used. The need for a more exacting criterion of freedom from infection in cases of Sonne dysentery was pointed out by the Emergency Public Health Laboratory at Oxford (*E.P.H.L.S.Bull.*, 1942) during an investigation of a small outbreak in children; they required 8 consecutive negative stools in 4 weeks or at least 6 negatives in 6 weeks before a case was discharged from isolation hospital. A few carriers may still be missed by these exacting tests; but less rigorous standards are certain to produce erroneous results and a position of false security owing to the intermittency of excretion of *Bact. dysenteriae* by carriers, particularly in the case of Sonne infections. It is admitted that the segregation of apparently healthy individuals for relatively long periods is a serious proposition, but such a measure is unfortunately essential in the case of any person involved in the handling or preparation of food, or in dealing with the disease in nurseries, schools, and mental institutions, where minor epidemics are prevalent and difficult to control.

The intermittency of excretion of *Bact. dysenteriae* also presents difficulties in the detection of carriers during an investigation of an outbreak. Unless several faecal examinations are made from each close contact it is possible for a carrier to escape detection. Therefore, when individuals are strongly suspected of being carriers, repeated tests must be carried out before a final opinion can be given.

The control of bacillary dysentery is thus a formidable proposition. The disease is widespread and, while many carriers already exist in the community, the number is likely to increase when troops return from service over-seas. Recent epidemiological studies clearly indicate that, unless the problem is tackled rigorously and a long-term policy adopted, hygienic control will have little effect on the incidence of the condition. Efforts to limit the disease by directing attention to personal hygiene and sanitation have not proved particularly successful, for, while these measures are simple and fundamental, they are difficult to enforce. These factors are without question of great importance in restricting transmission, but as long as the causative organisms are prevalent in a community they constitute a potential source of danger. Hardy and Watt (1944), during an extensive investigation of acute diarrhoeal disease in the U.S.A., found that dysentery bacilli could be isolated with ease from the fingers and from under the finger-nails of culturally positive cases and carriers.

Under active-service conditions over-seas the control of dysentery presents a much more difficult problem. The disease is often widespread in the native population, flies are abundant, hospitals are soon filled with cases, and, as laboratory facilities are relatively meagre, bacteriological control of the disease is impossible and is no longer attempted. In this country, particularly under normal conditions, the problem is much less

complex, but nevertheless careful consideration is necessary in deciding whether bacteriological control of bacillary dysentery is a practical proposition, because only thorough and intensive measures are likely to be successful. The main objections to embarking on an energetic campaign to control bacillary dysentery are: In this country the disease in adults is usually trivial, perhaps of comparable incapacitating value to the common cold, and many cases do not report for medical attention; many carriers exist; apparently healthy people may be segregated for relatively long periods; and the clinical course of the disease is readily controlled by sulphonamide therapy. It is, however, recognized that dysentery in infants, particularly undernourished infants, may be a serious and even fatal disease. Moreover, recent developments in laboratory technique have provided valuable information on the epidemiology of the disease and the detection of carriers has been greatly simplified; while the sulphonamides have proved extremely valuable in clearing all types of infection except Sonne. It should therefore be possible for us eventually to control bacillary dysentery provided all outbreaks are subjected to strict bacteriological control. Dramatic results must not be expected, as, in view of the wide distribution of the disease, its elimination from this country will undoubtedly prove a slow process.

Summary

The frequency of the carrier in bacillary dysentery and the intermittent excretion of *Bact. dysenteriae* have been readily demonstrated by the use of the desoxycholate-citrate medium.

Sulphaguanidine and succinyl-sulphathiazole have not proved successful in clearing persistent carriers of *Bact. dysenteriae* (Sonne).

The control of dysentery has been discussed and more stringent criteria recommended to determine freedom from infection—viz., 12 successive negative results.

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PLASMA ACID PHOSPHATASE IN CARCINOMA OF THE PROSTATE AND THE EFFECT OF TREATMENT WITH STILBOESTROL

BY

JEAN M. WATKINSON, B.Sc., M.R.C.S., L.R.C.P.

G. E. DELORY, M.Sc.

E. J. KING, M.A., Ph.D.

AND

A. HADDOW, M.D., D.Sc., Ph.D.

(From the Royal Cancer Hospital (Free), Fulham Road, and the British Postgraduate Medical School, London)

Kutscher and Wolbergs (1935) found large amounts of a phosphatase, having an optimum activity in the region of pH 5.0, in normal human prostate tissue and seminal fluid. Pursuing this investigation, Gutman, Sproul, and Gutman (1936) detected the enzyme in carcinomatous prostatic tissue and at the site of skeletal metastases secondary to carcinoma of the prostate.

Gutman and Gutman (1938) further observed an increase of "acid" phosphatase in the serum of patients with metastatic carcinoma of the prostate gland, and suggested that its estimation was of diagnostic value: patients with carcinoma of the prostate without bone metastases did not show an increase of acid phosphatase. Huggins and his collaborators (1941, a, b, c), at the University of Chicago then gave evidence that carcinoma of the prostate, in common with adult prostatic epithelium, is responsive to alterations in the level

of androgenic hormones in the organism. Injection of androgen caused a rise, and castration or injection of oestrogen a fall, in the plasma acid phosphatase. A study of the effect of castration and of oestrogen administration as a means of clinical management of advanced prostatic carcinoma showed that the improvement obtained was more than a palliation. Only 5% of the cases treated derived no benefit. Of the remainder, half showed a pronounced improvement, which was not, however, sustained for longer than 18 months. The others showed a more prolonged improvement.

The above work has been amply confirmed by other American authors: Sullivan *et al.* (1942) on the effect of castration; Kahle, Ogden, and Getzoff (1942) on the effect of stilboestrol; and Munger (1941) on that of x-irradiation of the testes. A review of the whole field has recently been given by Haddow (1943).

In preliminary investigations in these laboratories acid phosphatase estimations were performed on the plasma of normal controls, of patients with conditions other than carcinoma of the prostate, and of cases of carcinoma of the prostate with and without metastases in bone. Estimations were also carried out on normal human seminal fluid and in a case of eunuchoidism. In later work at the Royal Cancer Hospital more detailed investigations have been made on cases of carcinoma of the prostate and the effect of treatment with stilboestrol.

The cases now presented are unselected, although in one respect atypical in that they include a high proportion with osseous spread. No case was treated by x-irradiation of the testes or by castration, and in order to obtain clear results in cases in which the prostate had been irradiated previously, time was allowed for the full effect of this procedure to be assessed before oestrogen therapy was begun.

Method of Estimation of Acid Phosphatase: Principle

The pH optimum of acid phosphatase is between 4.5 and 5.0; that of alkaline phosphatase is between pH 8.4 and 10.0, depending upon the substrate used. Because of this great difference it is possible to estimate one phosphatase in the presence of the other merely by allowing it to act upon the substrate at its characteristic optimum pH. Thus the alkaline phosphatase in blood plasma is inactive at the reaction at which acid phosphatase operates best, and vice versa.

Method

The procedure for acid phosphatase determination is identical (and the solutions are the same, with the exception of the buffer) with that for alkaline phosphatase (see King, Haslewood, Delory, and Beall, 1942). An incubation time of 1 hour at 37° C. is normally used. The results are expressed in terms of units which are equal to mg. of phenol liberated in 1 hour. The calculation is carried out in the same way as for alkaline phosphatase. There are normally 1 to 5 arbitrary acid phosphatase units in 100 ml. of serum or plasma.

The colours are somewhat pale in the case of normal blood and in cases of carcinoma of the prostate in which the acid phosphatase is not greatly elevated, and it may be preferable in these instances to use a longer incubation period—e.g., 3 hours. If this is done the weight of phenol liberated (in mg.) is divided by 3 to reduce the figure to 1-hour unitage.

Solutions

Citric Acid-Sodium Citrate Buffer, pH 4.9—21 g. of crystalline citric acid are dissolved in water, 188 ml. of N NaOH added, and the solution made up to 500 ml. The pH should be checked, and adjusted to pH 4.9, if necessary, by dropwise addition of N NaOH or N HCl. This solution should be preserved with a few drops of chloroform and kept in the ice-chest. The other solutions are identical with those described by King *et al.* (1942).

Results

Acid Phosphatase in Plasma.—The values (Table I) for normal controls are somewhat higher than those given by Gutman and Gutman (1938), which range from 0.5 to 2.5 units per 100 ml. This may be explained by the fact that these authors prefer to employ a 3–5 hours' hydrolysis, and obtain a figure for 1 hour's hydrolysis by division.