(Himsworth and Scott, 1938a). The inference is that the substance responsible for insulin insensitivity is secreted by the pituitary gland. Clinically the evidence is provided by studies on the diabetes of cases of Cushing's syndrome and acromegaly. The diabetes associated with these two types of hyperpituitarism has been found to be of the insensitive type. It has been shown in such cases that

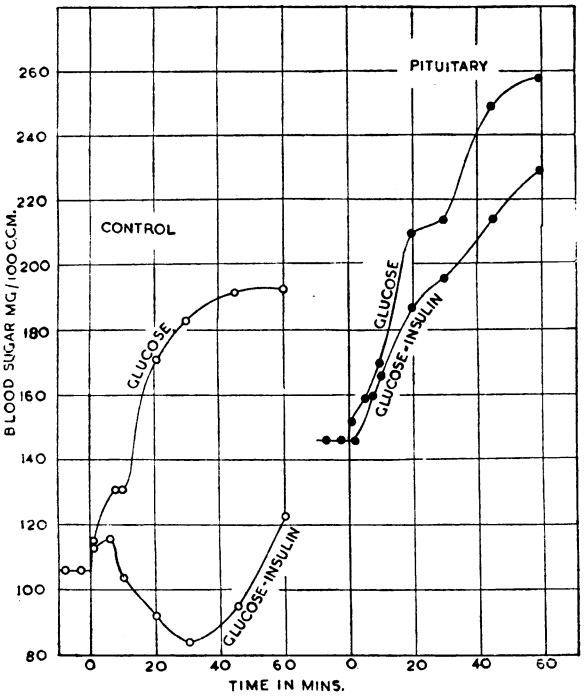

FIG. 4.—Glucose-tolerance and insulin–glucose curves from the same rabbit, before and after it had received injections of a crude extract of the anterior pituitary gland. In the untreated animal the fasting blood-sugar level is normal, the glucose-tolerance curve is normal, and the insulin–glucose curve is of the sensitive type. After the injection of the pituitary extract the fasting blood-sugar level is raised, the glucose-tolerance curve is at a high level, and the response to the insulin–glucose test is of the insensitive type.

irradiation of the pituitary region with *x* rays ameliorates the clinical severity of the diabetes and at the same time improves the sensitivity to insulin as measured by the insulin–glucose test (Himsworth and Kerr, 1939b).

In view of these facts it appeared profitable to attempt the detection of the anti-insulin type of pituitary factor in the body fluids of diabetics. No trace of any such substance was found in the blood of any case of diabetes mellitus. Neither was any trace found in the urine of sensitive or ordinary insensitive diabetics; but definite amounts were present in the urines of a case of acromegaly and two cases of Cushing's syndrome, and, further, the amount of anti-insulin substance excreted diminished with irradiation of the pituitary region (Himsworth and Kerr, in press).

Conclusion

It is convenient here to summarize the present position of our knowledge regarding human diabetes mellitus. There is considerable evidence that two types of diabetes can be differentiated on the basis of the speed with which they react to insulin. In one type, the insulin-sensitive type, insulin comes into action rapidly; in the other, the insulin-insensitive type, insulin comes into action slowly. The evidence is compatible with the suggestion that the disease in the sensitive type of case is due to deficiency of insulin, while in the insensitive type the disease is due not to lack of insulin but to impairment of insulin action. At present, although there is evidence that the anterior pituitary gland may be responsible for the diabetes associated with hyperpituitarism, the indictment of the pituitary gland as a primary factor in ordinary cases of human diabetes mellitus rests purely on analogy (Himsworth, 1939).

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STRAIN VARIATIONS IN THE RESISTANCE OF STREPTOCOCCUS VIRIDANS TO SULPHONAMIDE COMPOUNDS

BY

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The introduction of the sulphonamide group of drugs has once more brought into being hopes of a successful treatment of subacute bacterial endocarditis, and a number of papers on the application of these drugs to the disease have been published. Whitby (1938) reported two cases which were influenced by the use of sulphapyridine, and Ellis (1938) records one similar case and another influenced by sulphanilamide. Spink and Crago (1939) treated twelve fully authenticated cases of the disease, and in six of these sterility of the blood was attained, in four there was abatement of fever, and one case was apparently cured. Another case of apparent recovery is described by Andrews (1940).

Cases of Subacute Bacterial Endocarditis treated with Sulphonamides

A prominent feature of the treatment of subacute bacterial endocarditis is that some cases appear to be influenced by the drugs while others pursue their course quite unaffected by them. It seemed that the infecting organisms, though in each case a *Streptococcus viridans*, might differ in their susceptibility to sulphonamide derivatives. With this possibility in view four strains of *Strep. viridans* isolated by blood culture from patients suffering from subacute bacterial endocarditis were tested for their susceptibility to the bacteriostatic action of three drugs—4:4′-diaminodiphenyl sulphone, sulphapyridine, and the soluble sodium salt of sulphapyridine—their effects being compared with those produced by the drugs in the patient from whom the streptococcus was derived. The following are particulars of the four cases:

Case 1.—A bank clerk aged 32 had a five-months history beginning with a typical attack of acute rheumatism. A temporary improvement occurred, only to be followed by a

severe attack of haematuria accompanied by fever, which persisted, and the occurrence of fleeting pains in the shoulders and chest and of Osler's nodes. On admission to hospital the signs of mitral stenosis were present and the spleen was easily palpable. Blood cultures on two occasions yielded

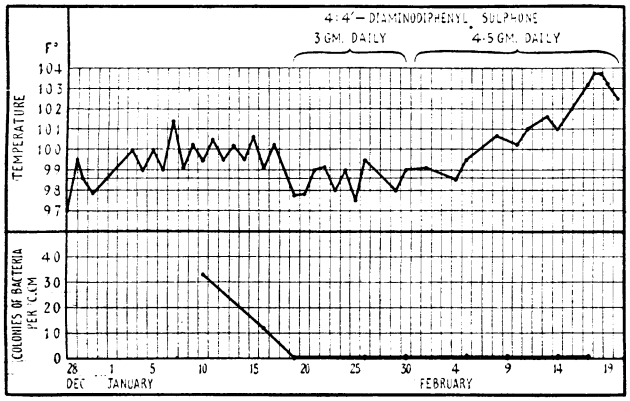

FIG. 1.—Temperature chart and bacterial counts in Case 1.

33 and 12 colonies of a *Strep. viridans* per c.cm. of blood. Treatment was begun with 4 : 4'-diaminodiphenyl sulphone, 1 gramme three times a day. A temporary decrease in the temperature occurred, though after two weeks of continuous treatment it gradually mounted again. The blood culture, however, became sterile and remained sterile on six subsequent occasions. Increased dosage of the drug produced no clinical improvement (Fig. 1). The patient died suddenly after thirty-three days of treatment, during which no positive blood cultures were obtained. At necropsy the heart showed severe healed rheumatic endocarditis of the mitral valve, in one area of which was a very small malignant vegetation at a site where one of the chordae tendineae had ruptured. Infarction of the spleen and focal embolic nephritis were also present. Histologically, serial sections of the mitral valve showed small areas of ulceration on the valve surface. In sections stained with haematoxylin and eosin no organisms could be seen, but in those stained by Gram's method many cocci were observed deep to the fibrin.

Case 2.—A nurse aged 39 had a six-weeks history of lassitude and fever. On admission to hospital she was febrile and a few petechiae were seen. Signs of aortic regurgitation were present in the heart. The spleen was not palpable. Blood cultures on two occasions yielded 34 and 39 colonies of a *Strep. viridans* per c.cm. of blood. Treatment was begun with 4:4'-diaminodiphenyl sulphone, 0.5 gramme four times a day, and continued for six days, when it was stopped on account of severe toxic symptoms. Sulphapyridine was then tried for two weeks, and again treatment was stopped on the occurrence of less profound toxic symptoms. Finally sulphanilamide was given for three weeks. Throughout treatment no decrease in the fever or clinical improvement was obtained, and the blood cultures remained consistently positive, with counts fluctuating from 92 to 29 colonies per c.cm. At necropsy large fleshy vegetations were found affecting a bicuspid aortic valve only. Splenic infarction and focal embolic nephritis were also present. Histologically, sections of the aortic valve showed innumerable cocci beneath the fibrin covering of large areas of ulceration.

Case 3.—A nurse aged 40 had a four-months history of the insidious onset of lassitude and fever, followed by the occurrence of petechiae and Osler's nodes. On admission to hospital she was febrile, and recent and fading petechiae were seen. The signs of mitral stenosis were present. The spleen was not palpable. Blood cultures yielded 208 colonies of a *Strep. viridans* per c.cm. of blood. Treatment with 4 : 4'-diaminodiphenyl sulphone, 0.5 gramme three times a day, was given for fifteen days. During the last ten days of this treatment the patient became afebrile and three successive blood cultures were sterile. Signs of toxicity appeared and the treatment was stopped, being started again with a smaller dosage. The blood culture became positive, and persisted so throughout the course of the disease. It was found that dosage of the sulphone above 1.5 grammes a day was accompanied by intolerance and that the fever continued unabated despite the treatment. The bacterial content of the blood varied between 20 and 40 colonies per c.cm. An attempt to raise the concentration of the sulphone in the blood by a continuous intravenous infusion of saline solution of it was made, and 16 grammes were given in sixty hours. A significant rise in the bacterial count from 18 to 107 colonies per c.cm. occurred immediately and there was no clinical improvement. Later treatment with sulphapyridine was also of no avail. Two weeks later the patient died as a result of a cerebral embolism (Fig. 2). At necropsy the heart was enlarged and the musculature was somewhat fibrotic. The mitral valve showed much thickening and shortening of its chordae tendineae, and there were many small friable vegetations confined to the cusps. Infarction of the lungs, spleen, kidneys, and brain was present. Histologically the valve cusps showed an ulcerated surface covered with fibrin, beneath which were innumerable cocci.

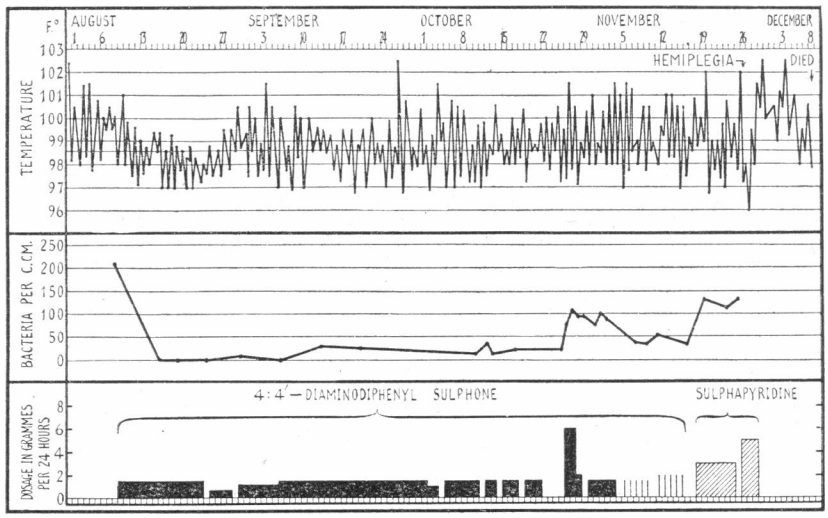

FIG. 2.—Temperature chart and bacterial counts in Case 3.

Case 4.—A garage proprietor aged 38 had a four-months history of fever and pains in the neck and shoulders followed by the occurrence of Osler's nodes. On admission he was febrile and a few petechiae were seen. There was marked pyorrhoea, and the signs of mitral and aortic regurgitation were also present. The spleen was not palpable. Blood cultures yielded a growth of *Strep. viridans.* Treatment was given with 4 : 4'-diaminodiphenyl sulphone, 0.5 gramme three times a day, for nineteen days. No clinical improvement or lowering of the temperature followed, and the bacterial count varied from 52 to 59 colonies per c.cm. of blood. Treatment with sulphanilamide, 2 grammes four times a day and later reduced to 1 gramme three times a day, was tried for ten days with similar results. Slight symptomatic improvement was obtained with two immuno-blood transfusions; but throughout the illness the pyrexia continued, and the bacterial count varied between 59 and 74 colonies per c.cm. The patient returned to his home and died two weeks later. No necropsy was made.

Bacteriological Technique and Findings

In these four cases frequent blood cultures were necessary and the following technique was adopted. Four glucose-broth tubes were inoculated with varying amounts

of citrated, or liquid containing, blood, and simultaneously three shake cultures, two with 1 c.cm. and one with 0.5 c.cm. of the blood, were made in melted legumin agar at 46° C. The broth cultures were incubated for at least fourteen days before a report of sterility was made. The three shake cultures were incubated, usually for forty-eight hours, and the average of the three plate counts was reported as the bacterial content of the blood.

The four strains of *Strep. viridans* obtained by blood culture from the cases described were tested *in vitro* against 4:4′-diaminodiphenyl sulphone, sulphapyridine, and the soluble sodium salt of sulphapyridine. The medium used throughout was a pancreatin digest broth of horseflesh adjusted to a *p*H of 8 as recommended by Okell. In this medium *Strep. viridans* produces a heavy flocculent growth, whereas in Wright's broth the growth is often scanty. Dilutions of each drug were made in the broth so that the resultant concentrations fell from 1 in 2,000 to 1 in 256,000. The technique was similar to that used by Francis (1938) in his work on susceptibility of *Brucella abortus* to sulphanilamide. To six series of dilutions were added known numbers of living organisms varying in amount from approximately 1,000,000 to 10. The actual size of each inoculum was determined by making shake cultures of the three final dilutions of the eighteen-hour-old broth culture of the streptococcus used for the experiment. The results of these tests are seen in Tables I and II.

An investigation into the fermentation reactions of each strain was made. The strains differed widely from each other, and marked morphological and colonial variations were observed. There appeared to be no relation between

TABLE I.—*Growth of Strep. viridans Strains isolated from Cases 1 to 4 in Broth containing Varying Amounts of Sulphapyridine*

Source of Organism	No. of Living Cocci in Inoculum	Dilution of Sulphapyridine in Broth								Control with no Drug
		1/2,000	1/4,000	1/8,000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	
Case 1 ..	200,000	0	0	0	+	+	+	+ +	+ +	+ + +
	20,000	0	0	0	0	0	0	+ +	+ +	+ + +
	2,000	0	0	0	0	0	0	0	0	+ + +
	200	0	0	0	0	0	0	0	0	+ + +
	20	0	0	0	0	0	0	0	0	+ + +
	2	0	0	0	0	0	0	0	0	+ + +
Case 2 ..	1,400,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	140,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	14,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	1,400	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	140	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	14	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Case 3 ..	700,000	+	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
	70,000	+	+ +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ + +
	7,000	0	0	+	+	+	+	+ +	+ + +	+ + +
	700	0	0	0	0	+	+	+ +	+ + +	+ + +
	70	0	0	0	0	0	+	+	+ +	+ + +
	7	0	0	0	0	0	0	+	+ +	+ + +
Case 4 ..	300,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	30,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	3,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	300	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	30	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

0 = no growth, + = slight growth, + + + = maximal growth in a forty-eight-hour culture.

TABLE II.—*Growth of Strep. viridans Strains isolated from Cases 1 to 4 in Broth containing Varying Amounts of 4:4′-diaminodiphenyl Sulphone*

Source of Organism	No. of Living Cocci in Inoculum	Dilution of 4:4′-diaminodiphenyl Sulphone in Broth								Control with no Drug
		1/2,000	1/4,000	1/8,000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	
Case 1 ..	2,800,000	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	280,000	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ + +
	28,000	+	+	+	+	+	+	+	+ +	+ + +
	2,800	0	0	0	0	0	+	+	+	+ + +
	280	0	0	0	0	0	0	0	0	+ + +
	28	0	0	0	0	0	0	0	0	+ + +
Case 2 ..	1,500,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	150,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	15,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	1,500	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	150	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	15	0	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Case 3 ..	2,000,000	+	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	200,000	+	+	+	+	+	+	+	+ +	+ + +
	20,000	0	0	0	0	0	0	0	0	+ + +
	2,000	0	0	0	0	0	0	0	0	+ + +
	200	0	0	0	0	0	0	0	0	+ + +
	20	0	0	0	0	0	0	0	0	+ + +
Case 4 ..	720,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	72,000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	7,200	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	720	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + +
	72	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + +
	7	0	+	0	0	0	0	0	0	+ + +

0 = no growth, + = slight growth, + + + = maximal growth in a forty-eight-hour culture.

the fermentation reactions and susceptibility to sulphonamide compounds. Table III shows these reactions.

TABLE III.—*Showing the Fermentative Reactions of the Four Strains of Strep. viridans*

	Case 1	Case 2	Case 3	Case 4
Lactose	+	+	+	−
Glucose	+	+	+	+
Saccharose	+	+	+	+
Maltose	+	+	+	+
Inulin	−	+	+	−
Salicin	+	+	+	−
Galactose	+	+	−	+
Raffinose	+	+	−	−
Mannite	+	+	−	−
Neutral red anaerobically	−	+	−	−
Litmus milk	A	AC	A	A
Morphology	Medium-length chain	Very long chain	Long chain	Short chain, ovoid cocci
Colonies	Small, semi-translucent	Small, semi-translucent	Small, semi-translucent	Minute
Haemolysis	α	α	α	α ; very slight
Heat at 60° C. for 30 min.	Killed	Killed	Killed	Killed

A = acid. AC = acid and clot.

Discussion

Strep. viridans is not a species but a heterogeneous group of cocci in many ways widely dissimilar. It is therefore not to be expected that all strains will behave similarly, and the data here presented show that they do not. The method used of testing bacteriostatic action in simple culture is open to the criticism that this effect is not known to be the same as that by which sulphonamide drugs exert their therapeutic effect in the body. If a test of therapeutic action in the experimental animal were available this would be more appropriate, but *Strep. viridans* causes no animal infection which can be used for such a test. A purely *in vitro* method is therefore the only means by which the action of sulphonamide compounds on this organism can be studied. If such a method reveals marked differences in strain susceptibility they must be of some significance. Very marked differences were in fact established, and these corresponded to the therapeutic findings.

Cases 1 and 3 were both cases in which the characters of the disease were apparently influenced by treatment with sulphonamide derivatives. In Case 1 the blood became sterile and remained so throughout thirty-three days of treatment until the patient's death. An interesting fact was the very small size of the vegetations found on the mitral valve at necropsy and the relatively small numbers of organisms which they contained. In Case 3 sterility of the blood was obtained for the first ten days of treatment only. The streptococci isolated from the blood of these two cases proved in the *in vitro* tests described to be susceptible to each of the three drugs tried.

Cases 2 and 4 were both cases in which sulphapyridine, 4 : 4′-diaminodiphenyl sulphone, and sulphanilamide were given intensively during treatment without any alteration in the manifestations of the disease. The streptococci isolated from the blood of these two cases proved to be resistant to the three drugs tested and grew plentifully in their presence.

Thus it appears that some strains of streptococci are resistant to and others susceptible to sulphonamide derivatives. It also seems likely that the strains of *Strep. viridans* which *in vitro* are resistant to these drugs are the causative organism in cases which fail to respond to treatment.

As regards the relative merits of each drug tested, there is little to choose between them, since each is effective against non-resistant strains. 4 : 4′-diaminodiphenyl sulphone is perhaps slightly the more potent *in vitro*, but this advantage is more than offset by the fact that this drug produces signs of intolerance, such as cyanosis, nausea, and vomiting, to a greater extent than does sulphapyridine. Sulphapyridine and its soluble sodium salt have almost identical inhibitory powers. The correlation of the results of the *in vitro* tests and the clinical course of these four cases is striking, but it must not be forgotten that periods of sterility of the blood are well recognized in subacute bacterial endocarditis and that the coincidence of such a period cannot be excluded in these cases.

The existence of strains of *Strep. viridans* resistant to sulphonamides is of importance when consideration is taken of the treatment used by Kelson and White (1939). This treatment comprises the oral use of sulphapyridine accompanied by the continuous intravenous infusion of heparin, and these authors claim good results in three out of six of their cases. This new treatment is as yet untried, but the success of it may well depend on the susceptibility of various strains of *Strep. viridans* to sulphapyridine. It might be worth while, before embarking on a course of treatment which is by no means free from risk, as well as difficult and costly, to determine the degree of susceptibility of the patient's streptococcus to the action of sulphapyridine by a cultural method like that used in these experiments ; a rather more simple form of experiment would probably serve this purpose.

Summary

Four cases of subacute bacterial endocarditis are described, two of which were apparently influenced by treatment with sulphonamide compounds, while the other two were quite unaffected by such treatment.

Simple *in vitro* tests are described in which it is shown that strains of *Strep. viridans* derived from these cases differ in their susceptibility to the three sulphonamide derivatives tried. The two strains isolated from cases which were clinically influenced by treatment were susceptible, whereas the strains isolated from the two cases which resisted treatment were also resistant to the drugs *in vitro*.

It is suggested that the existence of resistant strains of *Strep. viridans* may be responsible for the failure of some cases to respond to sulphonamide therapy.

Sulphapyridine is less toxic than 4 : 4′-diaminodiphenyl sulphone, and its activity only slightly less marked.

The use of shake cultures of the blood is recommended in following cases of subacute bacterial endocarditis.

I am greatly indebted to Professor L. P. Garrod for helpful advice and criticism, to Drs. A. E. Gow, G. Graham, and A. W. Spence for permission to publish cases under their care, to Miss G. E. Root for technical help, to Dr. S. Smith of the Wellcome Chemical Research Institute for supplies of 4 : 4′-diaminodiphenyl sulphone, and to Messrs. May and Baker for supplies of sulphapyridine (M & B 693) and its soluble sodium salt.

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Mr. W. H. Collins of Stoke Poges, chairman of King Edward VII Hospital, Windsor, has promised to give £20,000 towards the cost of rebuilding the out-patient department, installing new boilers, and erecting a restaurant for the staff. During the past eighteen months Mr. Collins has made two donations of £10,000 to the hospital.