atheroma. The blood cholesterol of the animals used was in the region of 500 mg./100 ml., compared with control levels of 80–120 mg./100 ml. Results were considered only in those animals which showed obvious macroscopic atherosclerosis in the aorta. A typical result is shown in Fig. 2. It can be seen that in spite of marked atherosclerosis the response to a single injection of benziodarone was similar to that obtained in normal animals, an increase in blood flow with a decrease in resistance and little change in blood-pressure being recorded in all six experiments.

FIG. 2.—Effect of single injection of benziodarone on myocardial blood-flow, blood-pressure, and resistance in a rabbit with experimental atheroma.

Discussion

Benziodarone has been shown to increase blood flow in the myocardium of the intact rabbit. The major increase in flow took place within 10 minutes, but slight increases above basal flow were noted in some animals for as long as 90 minutes. The systemic blood-pressure, by contrast with glyceryl trinitrate, showed only small changes, which suggests that the drug exerts a specific coronary vasodilator action in the doses used (5 mg./kg intravenously). The reason for such specificity is not clear, but it is not due to increased tissue oxidation in the myocardium (Charlier, 1961). We were unable to confirm in the rabbit the observations of Charlier that benziodarone favourably affects the E.C.G. changes after coronary artery ligation.

Many drugs which dilate coronary vessels have proved disappointing in clinical practice. One of the reasons may be that most drugs are examined on "normal" coronary vessels. In the present work we have used animals known to have atherosclerosis involving the coronary vessels and it was clear that benziodarone produced an increase in myocardial blood flow similar to that in normal rabbits. The correlation between animals with induced atherosclerosis and human disease must be viewed with care. There is certainly a difference in both the composition and the quantity of lipids in the plasma of normal humans and normal rabbits, but the plasma lipid pattern in atherosclerotic rabbits tends to become more like that found in human disease (Gofman *et al.*, 1950). There are also differences in extent and distribution of the pathological changes, for in the rabbit the atherosclerosis is more widespread.

Since the drug appears to have a specific dilator effect on the coronary vessels, and is able to induce coronary dilata-

tion in rabbits with experimental atherosclerosis, the results of clinical trials in this country should be of interest.

Summary

The effect of benziodarone on myocardial blood flow has been investigated, by means of a heated thermocouple technique, in normal rabbits and in rabbits with experimental atheroma. Intravenous benziodarone increased myocardial blood flow in both groups, without an effect on blood-pressure or heart rate. The main increase in myocardial blood flow lasted about 20 minutes, but smaller increases were found for longer periods. Benziodarone did not affect the E.C.G. pattern after ligation of a coronary artery.

Cardivix was kindly supplied by Genatosan Limited, who also generously provided a grant for expenses. We are grateful to Dr. Charlier for his advice and criticism in the preparation of this paper.

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DOUBLE-BLIND TRIAL OF BENZIODARONE IN ANGINA PECTORIS

BY

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Ideally any vasodilator drug used in the treatment of angina pectoris should have a powerful specific dilator action on the coronary arteries and should not increase the work of the myocardium disproportionately to the increase in coronary flow.

Of a series of more than 300 benzofuran derivatives tested on dogs and rabbits by Charlier and his associates (Deltour *et al.*, 1961), benziodarone (2-ethyl-3-(4-hydroxy-3:5-di-iodo-benzoyl)coumarone; "cardivix"; "amplivix") was found to be the most active coronary vasodilator. It fulfilled the above desiderata for such a drug and therefore has been studied in angina pectoris. The main work has been that of Gillot (1959), who observed a good effect in 77% of 180 subjects treated with benziodarone in an average dose of 300 to 400 mg. daily. Similar very favourable results have been reported by Dailheu-Geoffroy and Nataf (1961). Five double-blind trials have been published (Bekaert and Aubert, 1961; Vastesaeger, Rochet, and Leloup, 1961; Bredmose, 1962; Johnsen, 1962; Kloster, 1962), but with varying results.

In view of these findings we now report a double-blind trial of benziodarone against an inert control substance.

Material and Methods

Thirty-six patients (24 men and 12 women) took part in the trial. The ages of the men ranged from 40 to 78, and of the women from 38 to 80. All had ischaemic cardiac pain on exertion as diagnosed by the history. No attempt was made to assess the severity of angina pectoris, but its degree in all was such as to lead them regularly to attend a cardiac clinic. In 30 the story was supported by electrocardiographic changes; only five men and one woman had a normal electrocardiogram. No patient with anaemia or heart failure was included. One man had calcific aortic stenosis and one diverticulitis. Four men were receiving phenindione and this was continued. All were accustomed to use glyceryl trinitrate in an acute attack of pain.

A simple cross-over design was used, each patient being treated for a fortnight with benziodarone and a fortnight with the control substance, which was lactose. The two substances were prepared as 100-mg. tablets of identical appearance and dispensed in such a way that neither the patient nor the clinician could distinguish them. The dose was two tablets three times a day for the first four days, then one tablet three times a day for the remainder of the two weeks.

Patients were asked to continue to use glyceryl trinitrate for any acute attack of angina pectoris and record on a card each day the number of such attacks and their consumption of glyceryl trinitrate. They were requested also to note any side-effects.

Results

Of the 36 patients, three men failed to complete the trial: one, who was taking benziodarone, became depressed when his mother died; of the other two, who were taking lactose, one developed diarrhoea due to diverticulitis and the other jaundice associated with chronic alcoholism. A fourth patient, also male, suffered a non-fatal cardiac infarction while taking benziodarone and was removed from the trial. The Table shows the results of the trial in terms of the average number of attacks of angina pectoris and the average number of tablets of glyceryl trinitrate taken per day under the two regimes. As would be expected, there was close correlation between these two numbers for most

Analysis of Results (32 Patients)

	While Taking		Difference of Means	S.E. of Difference*	P†
	Benziodarone	Lactose			
Mean No. of attacks of angina daily	0.95	0.84	0.11	± 0.14	> 0.1
Mean No. of glyceryl trinitrate tablets taken daily	1.20	0.89	0.25	± 0.54	> 0.1

* Standard error calculated from individual differences.

† Probability based on t test.

patients. It is clear that, as judged by these criteria, there was no significant difference between benziodarone and lactose. Indeed, there were, on average, slightly more attacks of angina pectoris in patients when taking benziodarone than when taking lactose. There was a slight tendency, but again not significant, for patients to report more attacks in the first fortnight than in the second, regardless of the tablets taken. When asked at the end of the trial which set of tablets they preferred, five preferred benziodarone, nine lactose, while the remaining 18 could make no distinction. It seems highly likely that the preferences and differences in scores were the result of chance fluctuations, but we cannot, of course, exclude the possibility that a few individual patients may have benefited from the drug.

Such preferences as were expressed between the two substances agreed with the daily records of attacks kept by the patients, and were not affected by the presence or absence of side-effects. No serious side-effects were observed; in particular none of the four men receiving phenindione developed any haemorrhages. One man who was not having anticoagulant therapy had a severe haemoptysis but was found to be taking lactose. Of interest was the much reduced ^{131}I thyroid uptake caused by benziodarone in a man suspected of having thyrotoxicosis.

Discussion

Benziodarone has been claimed to be an effective and specific coronary vasodilator and to reduce the dependence of the patient on glyceryl trinitrate. Of the five double-blind trials published, in two (Bekaert and Aubert, 1961; Vastesaeger *et al.*, 1961) only 24% and 30% respectively of the patients showed no improvement when taking benziodarone. Similarly in a third trial (Johnsen, 1962) benziodarone was thought to be better than the placebo in reducing the frequency of angina pectoris. In the remaining two (Bredmose, 1962; Kloster, 1962) there was no significant difference between benziodarone and a placebo.

The trial now reported showed no significant difference between benziodarone and lactose (see Table). There were only seven occasions when a patient had more than two attacks of angina pectoris per day during a fortnight; five while taking benziodarone and two while taking lactose. No serious side-effects from benziodarone were observed. Although benziodarone may cause a reduction in prothrombin activity in those patients on long-term anticoagulant therapy (Bekaert and Aubert, 1961), no difficulty was experienced in controlling the dosage of phenindione. An important effect of benziodarone was the reduction of thyroid ^{131}I uptake resulting from the presence of two iodine atoms in its molecule (Bekaert, Deltour, and Broekhuysen, 1961).

Objective techniques for the assessment of drug therapy in angina pectoris have been introduced by Levy, Bruenn, and Williams (1940), Russek (1956), and Riseman, Altman, and Koretsky (1958). All are dependent on the resolution of electrocardiographic changes induced by exercise or hypoxia. Subjective methods depend on comparing the test substance against an apparently identical control substance by a double-blind technique as in the present trial. Even so the accuracy of data, whether obtained objectively or subjectively, is a function of the number and duration of such observations. It would appear that the ideal method would be for observers to work independently in a number of clinics and to report on the test substance on different groups of patients under varying conditions.

Our results confirm the previous experience of Oram and Sowton (1961), who commented that as yet no drug is available which can reduce the frequency of attacks of angina pectoris. Benziodarone is only one of a number of alleged long-acting coronary vasodilators which we have assessed. We have found none of any value and cannot but wonder whether the experimental and clinical search for a drug which dilates the coronary vessels may not be misguided. Thus drugs which are known to reduce cardiac pain quickly and effectively, such as glyceryl trinitrate and amyl nitrite, have no direct effect on diseased coronary vessels (Gorlin, Brachfeld, Messer, and Turner, 1959). Instead they act by reducing cardiac work as a result of a fall in systemic vascular resistance, following peripheral vasodilatation (Sharpey-Schafer and Ginsburg, 1962).

Summary

In a double-blind cross-over trial of benziodarone and lactose there was no significant difference between the two substances as measured by the average number of attacks of angina pectoris per day in a group of 36 patients.

We wish to acknowledge the help of our technicians in the Cardiac Department, King's College Hospital, and of Miss A. O. Gadsby in arranging the trial. Genatosan Limited kindly supplied and dispensed the benziodarone (cardivix) and lactose.

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QUANTITATIVE URINE CULTURES

BY

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Marple (1941) and Kass (1955) have described methods for determining the urinary bacterial content. In the present paper a quantitative culture technique is compared with routine non-quantitative urine cultures. The second part of the paper describes the relation between white-cell excretion and bacterial content in urines from patients with acute and chronic pyelonephritis and asymptomatic bacteriuria.

Methods

The urines were obtained from normal subjects and from patients with a wide variety of disorders, including many with urinary infections; no patient was being given antibacterial treatment at the time that the observations were made.

The "clean" samples of urine were collected without a catheter and the white-cell excretion rates were measured by methods previously described (Little, 1962). The upper limit of normal urinary white-cell excretion was taken as 400,000/hour.

Quantitative cultures were performed within one hour of collection, using a pour-plate technique. One millilitre of urine was taken from the clean specimen and tenfold serial dilutions from 1:100 to 1:100,000 were made in a phosphate buffer. One millilitre from each of these four dilutions was placed in a Petri dish and 10–15 ml. of cooled melted nutrient agar was poured on and mixed. The plates were incubated for 48 hours at 37° C. (Mackie and McCartney, 1960). The number of colonies was counted at the most convenient dilution. A further 10 ml. of urine was taken from the clean sample. This was sent immediately to the routine laboratory and dealt with as a ward sample. A loopful of uncentrifuged urine was placed on a MacConkey and blood-agar plate and incubated overnight at 37° C. If organisms were grown the culture was reported as positive in the following semi-quantitative terms: scanty growth, moderate growth, growth, or heavy growth. The organism grown was identified. It is generally considered that a quantitative count of more than 100,000 organisms per ml. is due to infection and that counts below 10,000 are due to contamination (Kass, 1955).

A diagnosis of acute pyelonephritis was based on a history of loin pain, frequency, and dysuria in a patient with fever and loin tenderness. The patients with chronic

pyelonephritis all had an unequivocal renal biopsy and/or intravenous pyelographic changes consistent with this diagnosis. A patient was considered to have asymptomatic bacteriuria when two urine samples contained more than 100,000 organisms per ml. and at the time of these cultures the patients did not have symptoms of infection of the renal tract. Some of these patients later developed acute pyelonephritis and are included in both categories at different times.

Results

Comparison of Routine Cultures with Quantitative Cultures

The comparison was made on 205 urine specimens: 134 contained fewer than 10,000 organisms per ml., "growth"

FIG. 1.—Routine urine culture report of "growth" and "no growth" compared with the number of organisms found on quantitative culture. The broken line is at 10,000 organisms/ml. and the unbroken line at 100,000/ml. The blocks of dots at the bottom of the figure represent counts of from less than 100/ml. to 2,000/ml., and at the top of the figure counts greater than 10,000,000/ml. Each dot represents one urine sample.

being reported in 53 of these (Fig. 1); 15 contained 10,000 to 100,000/ml., "growth" being reported in 13; and 56 contained more than 100,000/ml., the routine culture report being that of "growth" in all of these, but in four the