were poor. With successive injections of 400 μ g. the patients did not usually show the regular increment in excretion seen in normals (Fig. 4). The results in these patients were thus intermediate between those from normals and those from untreated patients with pernicious anaemia or steatorrhoea.

Discussion

Information derived from studies with vitamin B₁₂ has to some extent revised the premises on which the experiments now reported were based. Determinations of the potency of liver extracts and of the maintenance dose of vitamin B₁₂ lead to the conclusion that our group of patients under treatment for pernicious anaemia was not, in fact, especially resistant to treatment (Girdwood and Carmichael, 1950; Mollin, 1950). The possibility that vitamin B₁₂ may not be a complete therapy for pernicious anaemia still remains, but there is no evidence that such imperfections as have been reported can be corrected by P.G.A. (Beard, McIlvanie, and Nataro, 1950). During the course of these experiments, therefore, our interest has shifted to the more general problem of the metabolism of P.G.A. in health and in the megaloblastic anaemias, and this paper represents our preliminary observations in this field.

The excretion test used was necessarily not sensitive, and interpretation of the results is complicated by uncontrolled factors such as abnormalities of renal function. Nevertheless, the results enable one to speculate a little further on the abnormality of folic acid metabolism in patients with macrocytic anaemia; and the later findings, referred to above, have helped to eliminate some of the uncertainties. Folic acid probably exerts its therapeutic effect because the patients either have a deficiency of the substance or are unable to utilize it properly. Our results favour a deficiency. This could be absolute, due to deficient intake, deficient absorption, or increased excretion; or it could be relative, arising from increased need or increased destruction. Our results do not help in choosing between these possibilities except by excluding an increased excretion of folic acid. They merely indicate that in some patients with macrocytic anaemia the tissues have a great avidity for folic acid. In steatorrhoea, this could be due to an absolute deficiency, while in pernicious anaemia it might be the result of a relative or conditioned deficiency.

Summary

We have attempted to study the utilization of folic acid by measuring the excretion of small doses injected intravenously into four groups of subjects: 10 normal, 3 patients with steatorrhoea, 5 patients with untreated pernicious anaemia, and 13 patients with pernicious anaemia who were under treatment.

In normal subjects there was no increase in the excretion of folic acid in the urine when amounts of less than 200 μ g. of P.G.A. were given intravenously. There was an appreciable increase with 400 μ g., but the increase represented less than 10% of the injected dose. Hence utilization was good. The amount lost in the urine tended to rise with successive doses of 400 μ g.

The patients never excreted more of an injected dose than the normal subjects. Some did excrete less: 3 patients with steatorrhoea, 3 with untreated pernicious anaemia, and 2 treated patients with pernicious anaemia. Others failed to show a rising excretion with successive doses. There was an appreciable overlap between untreated pernicious anaemia and the normal, but all three cases of untreated steatorrhoea showed diminished excretion.

The results suggest that there may be a rapid and increased utilization of folic acid in some instances of megaloblastic anaemia. This implies a deficiency of folic acid, either real or conditioned, but the results do not enable one to say which. Possibly both occur in different groups of cases.

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THE USE OF PHENYLEPHRINE TO AID AUSCULTATION OF EARLY RHEUMATIC DIASTOLIC MURMURS

BY

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The detection of active rheumatic valvulitis still depends upon auscultation. As such valvulitis is often the only evidence of rheumatic carditis, its early recognition is important. Owing to the well-known difficulty in interpreting systolic murmurs, accurate diagnosis of aortic or mitral valvulitis depends chiefly on hearing an aortic or a mitral diastolic murmur. On first appearance these murmurs are faint, brief, and apt to be overlooked, especially when there is tachycardia. As is well known, mitral murmurs may be intensified by exercise and by certain postures, such as lying on the left side, and aortic murmurs by holding the breath at the end of expiration. These manœuvres seem to accentuate murmurs either by increasing the work of the heart or by bringing the site of turbulence closer to the stethoscope. It has been realized, however, that exercise may mask a mitral diastolic murmur during the period of tachycardia, and is helpful only when the observer concentrates on the phase of cardiac slowing that succeeds it. There seem to be two possible explanations for this: either that bradycardia facilitates auscultatory perception or that the period of slowing down after effort is associated with an increased mitral blood flow per beat. Whatever the explanation, it seemed reasonable to suppose that auscultation might be helped by a drug that slowed the heart rate and increased the stroke volume, such as a pressor agent

which caused carotid sinus slowing. A pressor effect was also desirable so that the efficiency of the aortic valve could be tested against an increased load.

On inquiry the drug that seemed most suitable for the purpose was *l*-1 (*m*-hydroxyphenyl)-2-methylamino-ethanol hydrochloride—"phenylephrine" hydrochloride (Boots). This is a synthetic sympathomimetic amine, described by the makers as having an action "intermediate between those of adrenaline and ephedrine." It causes peripheral vasoconstriction and consequent rise of blood pressure, followed by reflex bradycardia, and appears in some ways to resemble *nor*adrenaline. It may be administered by intramuscular or intravenous injection.

Present Investigation

A total of 118 injections were given to 64 patients, chiefly children, ten of whom were non-rheumatic. The dose was usually 0.25 mg. intravenously; but small children received 0.2 mg. and one heavy adult 0.4 mg.

The blood pressure rose sharply and reached its highest level within two minutes; it returned to normal four to eight minutes after the injection. The average increase of systolic and diastolic pressures was 38 mm. Hg and 25 mm. Hg respectively, the range being 10 to 75 mm. Hg for both pressures. The average decrease in pulse rate was 36, the range being 10-72, and the amount of fall being proportionate to the previous heart rate. Both blood pressure and pulse rate responses were similar in normal and rheumatic cases. Continuous electrocardiographic tracings showed that bradycardia first appeared 7-11 seconds after the injection and lasted three to four minutes. Ectopic beats were noted in 12% of cases and were associated with heart rates below 50; one patient developed intermittent nodal rhythm.

Each of the 64 patients was examined by at least two observers before and after phenylephrine. Repeated injections were given to the earlier cases to determine whether the responses were constant, and certain other patients were tested more than once in the course of their illness. Thus 30 patients received two or more injections. Of the 64 patients, 10 were suffering from conditions other than rheumatic fever and had normal hearts, 19 had rheumatic fever or chorea with no clinical evidence of carditis, and the remaining 35 had unquestionable rheumatic carditis.

In the 29 patients without carditis phenylephrine intensified the heart sounds, including the third heart sound, but did not produce aortic or mitral diastolic murmurs.

In patients with carditis phenylephrine greatly intensified any murmur (systolic or diastolic) that was already present, brought out murmurs hitherto undetected, and caused a number of vanished murmurs to reappear; moreover, any difficulty in distinguishing between a heart sound and a murmur was resolved. These 35 cases of rheumatic carditis fall into three groups.

Group 1.—Nine patients, in whom no diastolic murmurs had been detected previously, had such murmurs for the first time during the phenylephrine test (Table I). Five of

TABLE I.—Nine Patients Without any Definite Diastolic Murmurs Before Phenylephrine

Diastolic Murmur First Heard During the Phenylephrine Test	Additional Diastolic Murmur Heard Subsequently
4 aortic	2 aortic
3 mitral	1 mitral
2 mitral and aortic	1 mitral and aortic

them have not had the murmur since, but these are recent cases; the other four developed clinical diastolic murmurs subsequently. One of these cases in a recent rheumatic relapse has developed further carditis and is also included in Group 2.

Group 2.—Twelve children up to the time of injection had either an aortic or a mitral diastolic murmur, but not both (Table II). Immediately after phenylephrine, both aortic

TABLE II.—Twelve Patients With Only One Definite Diastolic Murmur Previously

Diastolic Murmur Preceding Phenylephrine	Additional Diastolic Murmur During Phenylephrine Test	Additional Diastolic Murmur Heard Subsequently
5 mitral	4 aortic, 1 none	2 aortic
7 aortic	3 mitral, 4 none	2 mitral

and mitral diastolic murmurs were heard in seven cases, and both murmurs were heard subsequently on routine examination in four of them. Of the five cases in which phenylephrine merely accentuated an existing murmur, only one, after a further seven weeks of rheumatic activity, produced a second diastolic murmur.

Group 3.—Fifteen patients in whom diastolic murmurs had disappeared were investigated within two weeks of that event (Table III). In eight cases both aortic and mitral

TABLE III.—Fifteen Patients With Previous Diastolic Murmurs, No Longer Heard Over Two to Three Weeks Before Phenylephrine

Previous Diastolic Murmurs	Diastolic Murmur Preceding Phenylephrine	Additional Diastolic Murmur During Phenylephrine Test
8 mitral and aortic {	4 mitral	4 aortic
	1 aortic	1 mitral
	3 no murmurs	1 aortic only, 2 aortic and mitral
6 aortic	6 no murmurs	5 aortic, 1 no murmur
1 mitral	1 no murmur	1 mitral and aortic

diastolic murmurs had been heard previously; four of these now had an isolated mitral diastolic bruit, one an isolated aortic diastolic bruit, and three had no murmurs. During the phenylephrine test both murmurs were again heard in seven of the cases, and one of the murmurs reappeared in the eighth. Isolated aortic diastolic murmurs had been heard previously in six cases, and five of these reappeared after phenylephrine. The fifteenth case originally had an isolated mitral diastolic murmur; in this instance phenylephrine produced both an aortic and a mitral diastolic murmur.

In addition 15 of these 35 patients were reinvestigated later, when rheumatic activity had subsided, and previously heard diastolic murmurs were no longer evident. When phenylephrine was given within four to five weeks of the disappearance of a diastolic murmur this murmur was again brought out; but if the interval was longer than five weeks no murmur could be detected after phenylephrine. In three of these 15 patients a single diastolic murmur, either aortic or mitral, had been present during the stage of activity, and phenylephrine had then brought out a second diastolic murmur never heard otherwise; such murmurs could not be heard after phenylephrine during convalescence in two of the cases. In the third case the artificially induced murmur could still be heard after phenylephrine during convalescence and then appeared at a later date spontaneously.

Effects of a distressing nature have been observed only in two cases. One migrainous subject complained of occipital headache for two hours, and the other patient described frontal headache and substernal oppression for two minutes. In neither was there an extreme increase of blood pressure, and after a second injection later the second subject was symptom-free. Apart from one instance of transient "dizziness," no other patient noticed any subjective

changes at all. A transient erythematous flush of the neck and upper chest was observed in four children, and was the only objective side-effect noted in the series.

Discussion

The murmurs brought out by means of phenylephrine usually became audible about three minutes after the injection, and commonly persisted for 15 to 25 minutes. As stated earlier, the maximum pressor response occurred within two minutes of the injection, and the blood pressure returned to normal within four to eight minutes. Bradycardia occurred as soon as the blood pressure began to rise—that is, about ten seconds after the injection—and lasted three to four minutes. It follows that aortic diastolic murmurs induced by the drug do not depend solely upon the height of the blood pressure and that induced mitral diastolic murmurs do not depend solely upon artificial bradycardia. In fact, aortic diastolic murmurs occasionally developed after the blood pressure and pulse rate returned to normal. It is possible that a damaged aortic valve may leak only after an increased load has been carried for a sufficient time; in other words, that valve strain depends not only upon the size of the load but also upon its duration. Indeed, the fact that the phenylephrine murmur may outlast detectable physiological changes has raised the question whether the drug itself might have caused further valve damage; but all such murmurs have been transient, and this possibility seems improbable.

Mitral diastolic murmurs induced by means of phenylephrine may also bear a similar delayed relationship to the known physiological effects of the drug. As they may still be heard clearly for several minutes after the heart rate has returned to the pre-injection level, their detection cannot be attributed to improved auscultatory perception at slow rates. An augmented mitral blood flow per beat, associated with an increased stroke volume, is more likely to be responsible for the murmur. Functional mitral diastolic murmurs in cases of patent ductus arteriosus and ventricular septal defect have been explained in this way. But the time lag again seems to demand the implication of some other factor.

An obvious possibility is that phenylephrine may increase the stroke volume by a direct stimulating action on the myocardium. At the time of writing only a few physiological studies have been made; these have revealed a rise of jugular venous pressure, right auricular pressure, and pulmonary artery pressure, associated with a slight fall in cardiac output and an increased stroke volume. The duration of these changes and their relationship to bradycardia have not yet been determined in our laboratory.

Summary and Conclusion

Phenylephrine, a sympathomimetic drug with a nor-adrenaline-like action, has been given to 64 patients, of whom 35 had rheumatic carditis.

Transient elevation of the blood pressure and reflex bradycardia occurred in like degree in both non-rheumatic and rheumatic cases with and without carditis.

Transient aortic and mitral diastolic murmurs were induced by means of phenylephrine in many cases of rheumatic carditis, but never in non-rheumatic patients or in cases of rheumatic fever without carditis.

Aortic diastolic murmurs so induced are attributed chiefly to an increased load from transient hypertension straining a damaged aortic valve, mitral diastolic murmurs mainly to

an increased mitral blood flow associated with an augmented stroke volume resulting from reflex bradycardia; but other factors appear to be implicated.

Such murmurs offer clear evidence of valve damage and may be of considerable diagnostic aid.

I am deeply indebted to Dr. Paul Wood for the original suggestion that initiated this work, as well as for all his subsequent encouragement and criticism. I would also like to express my gratitude to Dr. Eric Bywaters for his many helpful suggestions, and to Dr. Gerald Thomas and other colleagues who have assisted at the clinical observations.

EFFECT OF GROWTH HORMONE AND OF CHORIONIC GONADOTROPHIN ON PREPUTIAL GLANDS OF THE FEMALE RAT

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Selye (1951) has recently observed a striking increase in the size of the preputial glands of the female rat treated with growth hormone (somatotrophin, or S.T.H.). He stated: "Preliminary investigations have shown that under suitable experimental conditions, S.T.H. causes marked preputial-gland enlargement within 48 hours. Perhaps the sensitivity of this test might be further augmented by topical application of S.T.H. We mention these preliminary findings only because of the urgent need for a clinically applicable sensitive bio-assay method for the estimation of S.T.H. in biological fluids. Perhaps they will stimulate others to explore such possibilities."

We have been interested for some years in the question of whether an increase in the production of growth hormone by the anterior lobe of the pituitary gland could account for the well-known abnormalities of the foetus in diabetic and prediabetic pregnancies, as well as for the development of frank diabetes in women in the latter state (Barnes and Morgans, 1948; Gilbert, 1949; Barnes *et al.*, 1950). It seemed to us, therefore, that if, following Selye's suggestion, a simple and sensitive method for the assay of growth hormone could be based on the increase in the weight of the preputial glands of the female rat, it might be possible to test directly whether the urinary excretion, for instance, of growth hormone in pregnant diabetics was greater than that of non-diabetic pregnant women in comparable circumstances. As it was certain that chorionic gonadotrophin would be present in high concentration in the body fluids with which we should have to deal, it was important that the effects of this hormone on the preputial glands should also be studied, especially since evidence quoted in Selye's paper suggested that the effect might be far from specific for growth hormone. Mor-

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