contraction between the body of the left ventricle and the papillary muscles, which leads to mitral regurgitation, rapid decompression of the left ventricle, and concentric hypertrophy. The importance of outflow tract obstruction and the possible contribution of excessive catecholamines within the heart in producing an abnormally powerful and rapid contraction of the left ventricle are debated.

It is concluded that while lesions which produce eccentric hypertrophy do not usually involve the papillary muscles or cause subvalvar mitral regurgitation, disorders which cause obstruction to outflow and which are associated with concentric hypertrophy and with reduction in the size of the cavity in systole are often associated with papillary muscle disorder and thus with mitral regurgitation.

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Effect of Prostaglandin E_1 on Platelet Behaviour in Vitro and in Vivo

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Prostaglandins were first isolated by Goldblatt (1933) and von Euler (1934) from human seminal plasma and sheep seminal vesicles, and have since been found in other tissues (Bergström, 1965). One member of the group, prostaglandin E_1 (PGE_1), was shown to have very powerful vasodepressor activity, and in view of the close parallelism between the vasoactivity of compounds and their ability to affect platelet behaviour (Born *et al.*, 1965; Hampton *et al.*, 1967) it seemed likely that PGE_1 would be found to inhibit platelet aggregation.

Kloeze (1966) showed that in very low concentration PGE_1 could inhibit adenosine diphosphate (A.D.P.)-induced aggregation of rat, pig, and human platelets, and A.D.P.-induced glass adhesiveness of rat platelets. We report here some further effects of PGE_1 on aggregation induced by other agents, on glass adhesiveness in a whole-blood system, on the electrokinetic changes which A.D.P. and noradrenaline induce in platelets, and on the production of platelet thrombi in injured arteries in the rabbit.

Methods

Prostaglandin Solutions.—PGE_1 was dissolved in 96% ethanol to produce a stock solution of 100 μg./ml., which was stored at −20° C. The dilutions required for the various experiments were made by adding aliquots of the stock solution to normal saline.

Blood samples were taken into siliconized glass syringes from the antecubital veins of healthy volunteers or from hospital patients without acute illnesses or vascular disease. Then 9-ml. aliquots were transferred to siliconized centrifuge tubes containing 1 ml. of 3.8% trisodium citrate and gently mixed.

Platelet-to-glass adhesiveness in whole blood was measured by a modification of the Payling Wright (1941) technique (Emmons *et al.*, 1965a), and to assess the effect of PGE_1 0.1 ml. of normal saline or of PGE_1 in normal saline was added to 1.9 ml. of whole blood. Duplicate platelet counts were performed by the method of Brecher and Cronkite (1950) before and after rotating the 2-ml. samples in conical glass flasks for 40 minutes, and the fall in platelet count during rotation of the samples was expressed as a percentage of the initial count.

Platelet aggregation was studied by a modification of the Born (1962) optical density technique (Emmons and Mitchell, 1965). The optical density of platelet-rich plasma falls as aggregates form and rises again if they disperse. The fall

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in optical density at various stages of aggregation is expressed as a percentage of that produced by high-speed centrifugation. Platelet-rich citrated plasma (P.R.C.P.) was prepared by centrifuging whole blood at 200 g. for 10 minutes. 0.1 ml. of prostaglandin solution, of adenosine (Sigma), or of normal saline was added to 0.4 ml. of the P.R.C.P. before the addition of 0.1 ml. of aggregating agent. All experiments were carried out at 25° C. on P.R.C.P. less than one hour old.

The clumping agents used and their final concentrations were: A.D.P. 0.5 μg./ml. and 1.5 μg./ml.; noradrenaline 1.5 μg./ml.; 5-hydroxytryptamine (5-HT) 1.5 μg./ml.; adenosine triphosphate (A.T.P.) 15 μg./ml. (all from Sigma); connective tissue extract prepared by the method of Zucker and Borrelli (1962); and thrombin 0.2 unit/ml.

Platelet Electrophoretic Mobility.—P.R.C.P. was prepared, diluted with nine parts of platelet-poor plasma (P.P.P.), and the mixture brought into contact with non-siliconized glass as described previously (Hampton and Mitchell, 1966a, 1966b). Platelet electrophoretic mobility was measured at 25° C. in the horizontal capillary apparatus designed by Bangham *et al.* (1958), using a potential gradient of 2.66 V/cm. Mobilities were expressed as μ/sec./V/cm. PGE_1 solution was incubated with the P.R.C.P./P.P.P. mixture for 10 minutes at 25° C.; control tubes containing saline were also set up and handled in parallel. A.D.P. or noradrenaline dissolved in normal saline was then added to the mixture to give a final concentration of 0.05 μg./ml. (which in healthy subjects causes the maximal increase in mobility) and 0.5 μg./ml. (which causes a significant decrease in mobility).

Platelet Behaviour in Vivo.—Male grey chinchilla rabbits were anaesthetized with intraperitoneal urethane, and the cerebral cortical arteries were exposed (Honour and Ross Russell, 1962). Two types of arterial injury were inflicted with needle-pointed forceps as described by Honour and Mitchell (1964): (*a*) ***minor injuries*** which do not produce white masses of platelets within the lumen of the vessel spontaneously but do so when aggregating agents are applied to the site of injury, and (*b*) ***major injuries*** which breach the vessel wall, cause transient external bleeding, and then go on to form occluding intraluminal platelet masses (white bodies) which embolize and re-form over a period of several hours.

PGE_1 was either applied topically to the injured vessels from a pipette delivering 1 ml. in one minute or injected intravenously through an indwelling catheter in the jugular vein.

TABLE I.—*Platelet to Glass Adhesiveness With and Without PGE*$_1$

	% Fall in Platelet Count after 40 Minutes' Rotation										Mean	S.E.
Saline control	33	39	65	36	77	53	44	50	45	44	48·6	±4·05
PGE_1, 2·5 μg./ml. ..	16	21	21	14	24	24	0	40	8	32	20·0	±3·42

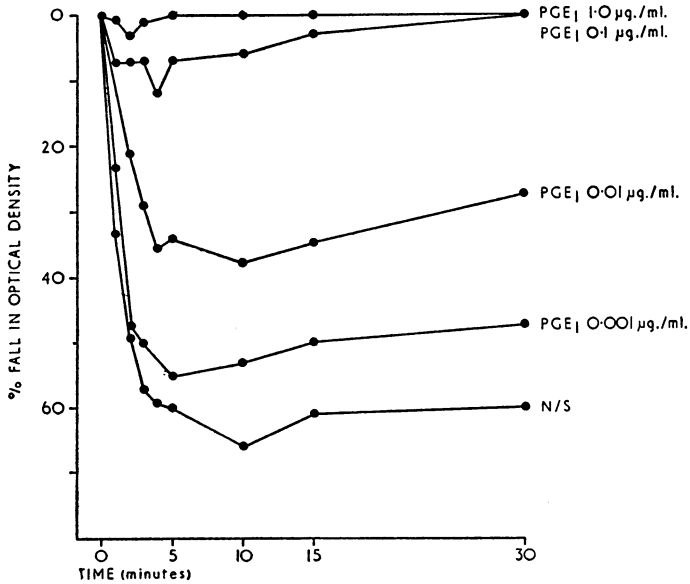

FIG. 1.—Percentage change in optical density produced by A.D.P. 0.5 μg./ml. in presence of various concentrations of PGE_1. N/S=normal saline control.

Results

Glass Adhesiveness

PGE_1 2.5 μg./ml. inhibited platelet adhesiveness in each of 10 experiments (Table I) and the mean adhesiveness was reduced from 48.6±4.0% to 20±3.4% ($p<0.001$). There was considerable patient-to-patient variation in this reduction of adhesiveness; with lower concentration of PGE_1 even more variable results were obtained, and in some patients adhesiveness was significantly reduced in the presence of as little as 0.01 μg. of PGE_1 per ml.

Platelet Aggregation

PGE_1 inhibited A.D.P. and noradrenaline induced aggregation. The degree of inhibition was proportional to the concentration of inhibitor over a range of 0.001 to 1 μg./ml. (Fig. 1). As in the adhesiveness studies, the effectiveness of PGE_1 varied considerably from subject to subject; in a group of six individuals PGE_1 1 μg./ml. reduced the mean maximum percentage change in optical density produced by A.D.P. 0.5 μg./ml. from 66 to 17% and by noradrenaline 1.5 μg./ml. from 66 to 8%. The inhibitory effect was enhanced by pre-incubating the prostaglandin with P.R.C.P. for 10 minutes before the addition of A.D.P., but incubation for longer periods of up to one hour had no further effect. When PGE_1 was added after aggregation had already occurred (Fig. 2) the aggregates dispersed rapidly.

The inhibitory activity of PGE_1 was compared with that of adenosine. In five experiments the mean maximum fall in optical density produced by A.D.P. 0.5 μg./ml. was 68% in the control tubes, 50% in the presence of adenosine 1×10^{-5} M, and 17% with PGE_1 2.8×10^{-6} M (1 μg./ml). PGE_1 is therefore a more powerful inhibitor of ADP-induced aggregation than adenosine.

PGE_1 (1 μg./ml.) also inhibited the aggregation produced by A.T.P., 5-HT, thrombin, and connective-tissue extract (Table II). It had no effect on the recalcification time of whole blood and P.R.C.P. or on kaolin cephalin clotting times. No morphological abnormalities were seen under the phase contrast

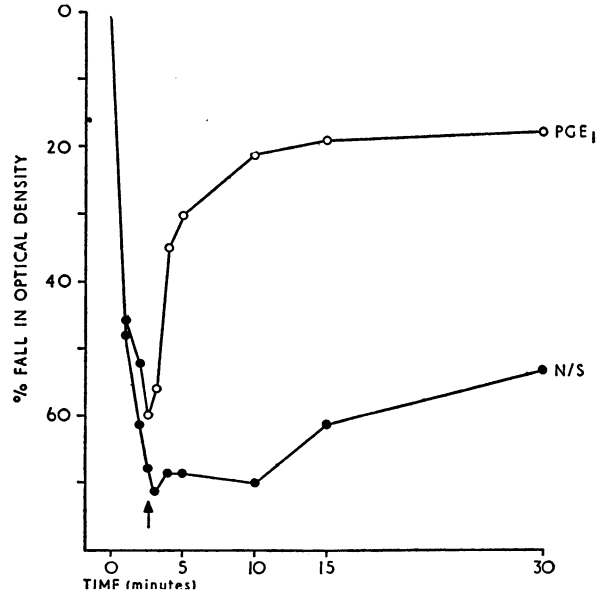

FIG. 2.—Effect of adding PGE_1 (final concentration 1 μg/ml.) and normal saline (N/S) two minutes after aggregation had been initiated by A.D.P. 0.5 μg./ml.

microscope of platelets from P.R.C.P. to which prostaglandin had been added.

TABLE II.—*Effect of PGE_1 on Platelet Aggregation in vitro*

Clumping Agent	Maximum % Change in Optical Density	
	Saline Control	PGE_1 1 μg./ml.
A.T.P. 15 μg./ml.	24	2
5-HT 1·5 μg./ml.	12	4
Connective-tissue extract	83	16
Thrombin 0·2 unit/ml.	25	0

Platelet Electrophoretic Mobility

(*a*) *Post-contact Mobility.*—PGE_1 in concentrations up to 1 μg./ml. had no effect on post-contact mobility.

(*b*) *Effect of A.D.P.*—Fig. 3 shows the effect of PGE_1 on the changes in platelet mobility induced by 0.05 and 0.5 μg. per ml. of A.D.P. The increasing and decreasing phases of the mobility change appear to have different sensitivities to PGE_1 in that 0.001 μg./per ml. of PGE_1 completely inhibited the decrease in mobility produced by 0.5 μg. of A.D.P. per ml. but had no effect on the increased mobility produced by 0.05 μg./ml. PGE_1 was also able to reverse the effect of A.D.P.: nine minutes after exposing the plasma mixture to 0.05 μg. of A.D.P. per ml. PGE_1 was added to give a final concentration of 1 μg./ml., normal saline being added to a control tube. The platelet mobility was measured as soon as possible, and in the control tube it had increased by 7% from its starting post-contact value, but in the tube to which PGE_1 had been added the increase in mobility was only 3%. Ten minutes later there was still a 4% increase in mobility in the control sample, but in the tube containing PGE_1 platelet mobility had returned to its initial post-contact value. The effect of added calcium on the action of PGE_1 was studied in the presence of heparin 2 u./ml., which

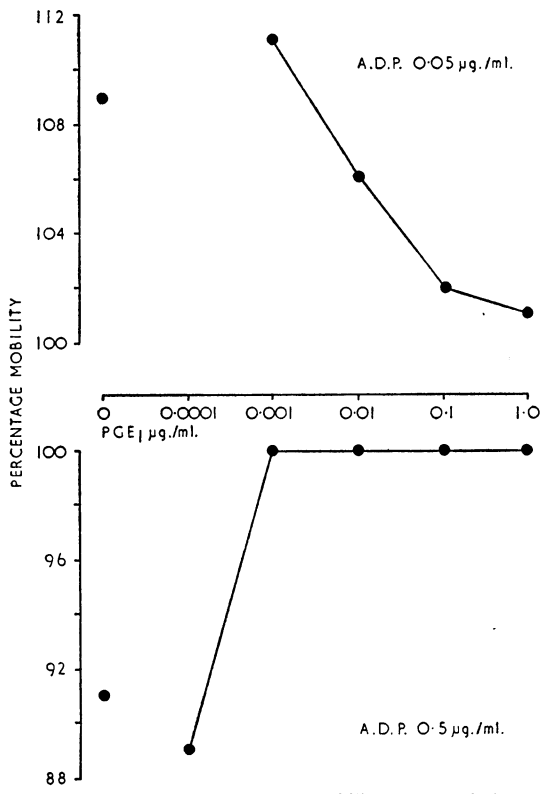

FIG. 3.—Platelet electrophoretic mobility changes induced by A.D.P. in presence of various concentrations of PGE_1. Upper section shows that 1 μg./ml. prevents the increase in mobility produced by A.D.P. 0.05 μg./ml., while the lower section shows that 0.001 μg./ml. can prevent the decrease in mobility produced by A.D.P. 0.5 μg./ml. Mobility values are expressed as a percentage of the basal. post-contact mobility.

itself was shown to have no influence on the platelet response to A.D.P. or on the effects of PGE_1. It was found that the addition of calcium chloride to give a theoretical increase in Ca^{++} concentration of 25 mM completely abolished the effect of 0.001 μg. of PGE_1 per ml. on platelets challenged with 0.5 μg. of A.D.P. per ml.

(*c*) *Effect of PGE_1 on the Response to Noradrenaline.*—PGE_1 inhibited the changes in mobility induced by noradrenaline in the same way as those induced by A.D.P.: the decreasing phase of the response was more sensitive to PGE_1 than was the increasing phase. However, both phases of the mobility response to noradrenaline were 10 times less sensitive to PGE_1 than were the mobility responses to A.D.P.: 0.01 μg. PGE_1 per ml. had no effect on the changes induced by 0.05 μg. of noradrenaline per ml., and 0.001 μg. of PGE_1 per ml. had no effect on those induced by 0.5 μg./ml.

In-vivo Studies

(*a*) *The Topical Application of PGE_1* to minor injury sites and to uninjured cerebral cortical arteries in concentrations of 2–40 μg./ml. did not produce platelet thrombi. Major injuries were then inflicted, and in each case white bodies began to form at the injury sites, embolizing every two to five minutes. PGE_1 2–10 μg./ml. was then dripped on to the cortex, 1 ml. being applied in one minute. No effect was observed with 2 μg./ml., but with 5 and 10 μg./ml. white-body formation stopped during the application and did not restart for 5 to 10 minutes. The sites then became spontaneously active, forming white masses which embolized.

(*b*) *Intravenous Injection of PGE_1 After Injury.*—Major injuries were inflicted in a series of rabbit preparations, and, after they had produced large regularly embolizing platelet masses for at least 20 minutes, PGE_1 in 5 ml. of saline was given over the course of one minute into the jugular vein cannula. Table III summarizes the results and shows that in our preparations 5 μg. was ineffective, that 10 μg. was a marginally effective dose which affected white bodies only while the injection was proceeding, and that 25 μg. or more would inhibit white-body formation. In some of the preparations we observed that a site which had become completely quiescent after intravenous PGE_1 might begin to form tiny translucent white bodies some 20 to 40 minutes after the PGE_1. This activity would last for 5 to 10 minutes, when the site would again become inactive, remaining so throughout a further two-hour period of observation. We have also seen this pattern of behaviour in rabbits given adenosine and dipyridamole ; it may represent a build-up in the circulation of a new population of platelets which have not as yet been exposed to the inhibitors, but its precise significance is not clear.

(*c*) *Intravenous Injection of PGE_1 Before Injury.*—In the rabbits which had received PGE_1 intravenously new major injuries were inflicted 30 to 40 minutes later. These produced small translucent emboli for a very short period (8 minutes with the 1,000-μg. dose ; 20 minutes with 100 μg. and 10 minutes with 50 μg.) and then became inactive. Injuries inflicted 60 to 80 minutes after the PGE_1 produced larger and more dense emboli, but these were also short-lived in the animals given the larger doses (15 minutes with 1,000 μg. and 12 minutes

TABLE III.—*Effect of Intravenous PGE_1 on White Body Formation in Injured Cerebral Arteries in the Rabbit*

Dose		Effect
μg.	μg./kg.	
1,000	312	White-body production stopped within 1 minute of injection
100	34·5	
50	15·1	White body washed away during injection. Then phasic production of tiny masses for 18 minutes, when site became inactive
25	9·3	White body washed away during injection. Phasic activity for 8 minutes, then became inactive
10	4·1	White body did not grow during injection. Thereafter, white masses produced in normal way
5	1·9	No effect

with 100 μg.). In the animals given 50 and 25 μg. these late injuries showed the same response as in a normal animal, and formed dense white masses for as long as the observation was continued.

(*d*) *Intravenous Infusion of PGE_1.*—Two rabbits with active white-body-producing major injuries were studied. The first animal was given PGE_1 in normal saline via a jugular vein cannula, the drip-rate being adjusted so as to provide 0.2, 0.4, 0.8, and 1.6 μg./kg./min., each rate being maintained for 15 minutes. At the lowest infusion rate white-body formation continued unabated, but at 0.4 and 0.8 μg./kg./min. the white bodies forming at the site washed away in showers of tiny fragments. The residual mass at the site continually changed its shape and size, but was unable to grow across the lumen and did not give rise to discrete emboli. When 1.6 μg./kg./min. was infused the residual mass became progressively smaller and after 13 minutes the site became inactive. In the second animal the initial PGE_1 infusion was at a rate of 0.8 μg./kg./min. for 15 minutes, and the white mass at the injury site began to wash away as fast as it formed, showers of minute fragments swirling away from its periphery. During a 15-minute period at 1.6 μg./kg./min. the mass washed away more rapidly than it formed and became very small. Seven minutes after increasing the infusion rate to 3.2 μg./kg./min. the site became inactive. When the 15-minute infusion period at this rate was completed normal saline was substituted and the site immediately became active again, remaining so for 23 minutes. There was then a further 50-minute period of inactivity, after which white bodies formed until the animal was killed. As the prostaglandins are inactivated in the lungs (Bergström and Samuelsson, 1965) intra-arterial infusions may prove to be even more active in modifying white-body formation, and we are now examining the effect of intracarotid PGE_1.

Discussion

Kloeze (1966) showed that PGE_1 was a powerful inhibitor of A.D.P.-induced platelet aggregation in human, rat, and pig plasma, but found it to be without effect on platelet-to-glass adhesiveness in a platelet-rich plasma system to which A.D.P. had not been added. We have confirmed the effect of PGE_1 on A.D.P.-induced aggregation in man and have shown that it inhibits aggregation by other agents. We have also found that it inhibits platelet-to-glass adhesiveness when tested in a whole-blood system, that it modifies the electrokinetic response of platelets to A.D.P. and to noradrenaline, and that it inhibits the formation of platelet masses in injured rabbit cerebral cortical arteries.

In some of its effects on aggregation and adhesiveness PGE_1 resembles other vasoactive compounds (Hampton *et al.*, 1967), but there are some significant differences. First, PGE_1 is active at much lower concentrations than any other agent so far tested, and, second, like adenosine but unlike the other vasodilators, pre-incubation for 10 minutes enhances the inhibitory effect of PGE_1 on aggregation. PGE_1 also resembles adenosine in that it reduces the initial rate of aggregation and the maximum degree of aggregation attained, while other vasodilators which we have tested have little effect on these early phases of aggregation but markedly enhance disaggregation. The way in which adenosine itself acts is not known; it was thought to act as a competitive inhibitor of A.D.P. (Born and Cross, 1963), but the 10-minute lag before maximal inhibition, and the lack of inhibitory effect in species such as the cat and rat which nevertheless respond to A.D.P., suggest that we must seek an alternative explanation of its action.

In its ability to inhibit the electrokinetic response of platelets to A.D.P. and noradrenaline, PGE_1 also resembles other vasoactive agents. However, PGE_1 proved capable of influencing the platelet charge characteristics at much lower concentrations than other known inhibitors, and also proved to be more active against A.D.P. than noradrenaline. We found that the addition of Ca^{++} ions counteracted the inhibitory effects of PGE_1 and that PGE_1 was more active against the decrease in mobility produced by higher concentrations of A.D.P. and noradrenaline than against the increase in mobility produced by lower concentrations. This closely parallels the E.D.T.A. sensitivity and calcium requirement of the two phases of the mobility change, the decrease in mobility being more calcium-demanding. It seems possible that the action of PGE_1 on platelets may be mediated by alterations in their Ca^{++} transport mechanism. We have already drawn attention to the structural and functional similarities between arterial smooth-muscle cells and platelets (both have a contractile protein with Ca^{++}-dependent A.T.P.-ase activity, and both show a common sensitivity to vasoactive agents). It is of interest that Coceani and Wolfe (1966) have suggested that the action of the prostaglandins in initiating contraction in the smooth muscle of the rat stomach is mediated by the facilitation of calcium influx or the release of bound calcium.

The mechanism of action of PGE_1 in the in-vivo preparation is also unknown. Other vasodilators are capable of inhibiting white-body formation, but there is no direct relation between their activity on thrombus formation in vivo and their ability to inhibit platelet aggregation in vitro. This lack of correlation and the activity of many of the compounds when applied locally suggests that some of them are acting on the injured artery wall cells rather than on the circulating platelets. For example, dipyridamole has little effect on platelet aggregation in vitro but has a pronounced inhibitory effect on white-body formation when given intravenously or when applied locally (Emmons *et al.*, 1965b). In contrast, PGE_1 is a powerful inhibitor of in-vitro platelet behaviour, and in the rabbit is more active intravenously than when applied topically. It is likely, therefore, that it acts by an effect on the circulating platelets, and it is of interest that in the animals given PGE_1 there was no evidence of defective haemostatic plug formation.

The physiological role of prostaglandins is unknown. They are thought to be biosynthesized from arachidonic acid and homo-γ-linolenic acid, both of which have essential fatty acid activity. Early reports that linolenic acid itself could inhibit platelet adhesiveness (Owren *et al.*, 1964) could not later be confirmed (Borchgrevink *et al.*, 1965, 1966). There is no evidence that the essential fatty acids themselves have any of the properties which we have shown PGE_1 to possess, nor does it seem likely that the linolenic acid tested by Owren *et al.* (1964) contained prostaglandins.

It is not known whether any of the aspects of platelet behaviour which we have investigated are relevant to the pathogenesis of thrombotic arterial disease, but the ability of a biological material such as PGE_1 to exert profound effects on the blood platelets both in vitro and in vivo at such low concentrations makes it imperative to study its activities in greater detail.

Summary

Prostaglandin E_1 (PGE_1) is a powerful inhibitor of platelet-to-glass adhesiveness in a whole-blood system and of in-vitro platelet aggregation induced by A.D.P., noradrenaline, A.T.P., 5-HT, and connective-tissue extract. PGE_1 also inhibits the changes in platelet electrophoretic mobility produced by A.D.P. and by noradrenaline.

PGE_1 is active in vivo, for topical applications and intravenous injections or infusions suppress platelet thrombus formation in injured rabbit-brain arteries.

PGE_1 is found in many organs, and as it is capable of modifying platelet behaviour in vitro and in vivo in very low concentrations its role in the natural history and treatment of arterial thrombosis merits further study.

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Vi Reaction in Hong Kong

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After 30 years the value of the Vi reaction remains controversial (Public Health Laboratory Service Working Party, 1961; Bokkenheuser and Richardson, 1964), and several factual points in connexion with the test are still in doubt. As typhoid fever is endemic in Hong Kong, it was decided to conduct an investigation into the use of the Vi reaction in the diagnosis and epidemiology of typhoid under the rather unusual conditions pertaining in the colony.

Before any work could be done on the place of the Vi reaction in the detection of typhoid carriers it was essential to ascertain the normal range in the general population.

In the study of the acute phase of typhoid an attempt was made to establish whether the Vi reaction could be an aid to the serological diagnosis of the disease, special emphasis being given to the possibility of forecasting clinical relapse or the development of the carrier state. The records were also examined for correlation between Vi titres and the following: age, severity of disease, Widal reaction, and treatment.

Method and Materials

Blood was collected for Vi examination from three groups of subjects: group 1, 100 orthopaedic patients and prisoners with negative histories for typhoid and T.A.B. vaccination; group 2, 75 patients with culturally proved typhoid on whom Vi examinations were carried out at weekly intervals during their stay in hospital, and then, so far as was possible, at three-monthly intervals up to 12 months; and group 3, 17 proved typhoid carriers.

Serological Examination

The Vi antibody test was carried out with a suspension prepared from the Colindale Vi suspension (Colindale Public Health Laboratory, London) and the standard Vi sera from Colindale as the reference sera. The prepared Vi antigen suspension was tested at regular intervals with the titrated standard sera and in conjunction with the sera under test. The titre of the test sera was taken as the reciprocal of that dilution of the sera giving the same degree of agglutination as that of the standard sera at a dilution of 1,600 set up at the same time.

The test itself was performed by adding 0.3 ml. of the antigen suspension to 0.3 ml. of the test sera diluted in twofold steps from ½ to 1/64, giving final reciprocal serum concentration from 4 to 128, further doubling dilution being set up later if necessary. Both test and controls were incubated for two hours at 37° C. and then overnight in the refrigerator.

In reading the test the three standard diagnostic criteria were used to assess the degree of agglutination: the clearing of the supernatant, the pattern of the deposit, and the resultant granularity after agitating the tube.

Results

All Vi estimations were carried out by one of us (M. J. R.).

Normal Levels

Sera were obtained from 100 subjects representative of the main economic strata in Hong Kong, comprising orthopaedic patients and prisoners, none of whom gave a history of enteric-like infection or of T.A.B. immunization. The results of this sample are shown in Table I, which also gives the titres for those in the group who were under the age of 20.

The upper limit of normal was chosen as 32. This leaves 4% of the population as positive reactors. (At the British level of 5 as positive the Metropolitan Water Board found 3.2% positive reactors (P.H.L.S.W.P., 1961).)

TABLE I

Vi Titre:	Neg.	4	8	16	32	64	
Total No. of subjects ..	7	16	22	24	27	4	100
No. under 20	—	4	1	3	3	1	12

Acute Disease

The patients consisted of 75 unselected consecutive cases admitted to a Government hospital with typhoid fever. Before a case was accepted into the series it was necessary for *Salmonella typhi* to have been recovered from the blood or faeces. Apart from the exclusion of treated patients transferred late in the disease from other hospitals there was no selection. The sex incidence was equal, being 38 males and 37 females.

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