ing may be avoided, and an occasional laparotomy may be prevented.

4. The treatment is either medical or surgical.

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408 Birks Bldg.

RÉSUMÉ

L'auteur passe en revue les différents cas de "slipping rib syndrome" qu'il a relevés dans la littérature médicale et rapporte lui même un cas dans son expérience. Il déplore le fait que l'on n'attache pas assez d'importance à ce syndrome dans le diagnostic différentiel des affections douloureuses thoraciques et abdominales. Le diagnostic en est simple et il pourra parfois éviter une laparotomie inutile. Le traitement est chirurgical et médical. YVES PRÉVOST

A NEW PROTHROMBOPENIC DRUG, PHENYLINDANEDIONE*

L. B. Jaques, M.A., Ph.D., E. Gordon, B.A. and E. Lepp, B.A., R.T.

Saskatoon, Sask.

WHILE dicoumarol has come into widespread clinical use in virtue of its marked ability to reduce the prothrombin content of the blood, anticoagulant therapy with this agent is not ideal because of the slowness of recovery of blood prothrombin levels after administration of the drug has ceased. A definite demand exists for some agent which has the valuable properties of dicoumarol in its effect on prothrombin but which is followed by a more rapid recovery from the effects of the drug.

In 1947 in the course of a study of the relation between structure and anti-vitamin K activity, Meunier, Mentzer and Molho[1] drew attention to the drug phenylindanedione, which showed a transitory effect on prothrombin levels. Soulier and Gueguen[2] later reported on the effect of single doses of this drug. They reported that the drug, in both the experimental animal and in patients, was effective in lowering the prothrombin level, although the doses used (10 to 20 mgm./kg.) were higher than the effective dose of dicoumarol. However, in all cases, the drug caused a more rapid fall in prothrombin, and a more rapid recovery. As this drug does not appear to have been brought to the notice of workers in the U.S.A. and Canada, we[3] have investigated it experimentally and have found that the drug differs from dicoumarol in its action in several important respects, as reported below. An accompanying clinical study of the drug is reported by Dr. A. Blaustein in this number.

METHODS

Prothrombin times.—As emphasized by Link,[4] the procedure of Quick is not satisfactory for following the action of dicoumarol. In order to determine small changes in the prothrombin system, it is advisable to modify the method by using diluted plasma. Jaques and Dunlop[5] have shown that after dicoumarol, the prothrombin time becomes very sensitive to changes in calcium concentration, and hence this factor also may be used to make the system a more sensitive indicator of the effects of prothrombopenic agents. In our preliminary studies, we have used therefore a concentration of 0.004 M calcium. In all other cases, we use 0.02 M calcium chloride. Prothrombin times were determined on the plasma itself and also frequently after dilution to 50, 25 and 12.5%. The thromboplastin used was a saline extraction at 53-55° for 15 minutes, of acetone-dried rabbit brain powder. The animals received the ordinary laboratory diet, consisting chiefly of fox chow cubes.

The 2-phenylindanedione-1, 3 was prepared by Dr. A. D. Odell, of the Charles E. Frosst and Co., Montreal, to whom we are greatly indebted. The drug was given orally in gelatin capsules.

RESULTS

Effect of single doses of P.I.D.—To compare the effect on prothrombin time of P.I.D. with dicoumarol, single doses of each drug were given at intervals to a series of dogs and rabbits. Typical curves are shown in Fig. 1. It can be seen that the prothrombin time reached a peak value of 21 to 23 seconds 24 hours after the anticoagulant was administered. However, while after dicoumarol, the prothrombin time took approximately three days to return to normal, after P.I.D., recovery was complete in 14 hours. The prothrombin time after P.I.D., as after dicoumarol (not shown) begins to rise to the peak value after the 10th hour, showing a lag due to the time required for absorption and for its action on the liver(?). However, a definite difference is that P.I.D. shows an effect on the prothrombin time immediately after administration, giving rise to a raised prothrombin time for two to three hours. This effect passes off and is succeeded by the "dicoumarol" effect. This preliminary peak has been observed consistently. As will be reported elsewhere, P.I.D. in comparable con-

* From the Department of Physiology, University of Saskatchewan, Saskatoon, Sask., Canada. Aided by a grant from the National Research Council of Canada. Phenylindanedione (Danilone) was manufactured and supplied by Charles E. Frosst & Co., Montreal.

centrations does not affect the prothrombin time when added to the blood *in vitro,* so at present we have no explanation for this finding. The prothrombin time values below normal (hypercoagulability) seen in Fig. 1 with dilute plasma were not seen in most experiments and did not occur more frequently than with dicoumarol.

Since the same peak prothrombin time was obtained with 6 mgm. of dicoumarol as with 50 mgm. P.I.D., this suggests that P.I.D. has only about one-tenth the potency of dicoumarol. To test this, the effect of various dose levels of P.I.D. on prothrombin time was studied (Table I).

Examination of this table suggests that there is relatively no difference in the prothrombin time values obtained with different doses of P.I.D. Thus, rabbit 11 showed the same response for doses of 25 and 100 mgm. In contrast, doubling the dose of dicoumarol at these levels, approximately doubles the prothrombin time.[6] With P.I.D. the difference in response between different animals while not marked had a greater effect than differences in dosage level. To our surprise, in several experiments, with doses of 200 mgm./kg. and 20 mgm./kg. in these same animals, no effect was observed on prothrombin time. While blood samples were not taken at sufficiently frequent intervals so that one could say that no change had occurred, in view of the results obtained with intermediate doses, it is surprising that we did not observe some change in the value.

These results are not in agreement with the conclusions of Soulier and Gueguen, who concluded that there was a direct relation between dosage of P.I.D. and the prothrombin time produced. Inspection of the curves reported by Soulier and Gueguen, however, also fails to demonstrate the clear direct proportionality between dosage and effect on prothrombin time observed with dicoumarol.

In view of Jaques and Lepp's demonstration that salicylate has a prothrombopenic action only when given by mouth,[7] P.I.D. was given intravenously to several animals. It was evident that the drug was equally effective by vein and that its action did not depend on the contents of the gastro-intestinal tract as does sodium salicylate. These results indicate that P.I.D. is a weak prothrombopenic agent compared to dicoumarol, but recovery of the prothrombin time is faster following the administration of P.I.D. than of dicoumarol. Meunier[8] has suggested that our failure to confirm the marked effect on prothrombin time with these doses is due to the prothrombin time method used by him being different to the Quick test.

Effect of repeated doses of P.I.D.—In view of the transitory nature of the effect of P.I.D. the effect on prothrombin time of repeated

TABLE I.

EFFECT OF SINGLE DOSES OF P.I.D. ON PROTHROMBIN TIME

Subject	*Dosage mgm./kg.*	*Prothrombin time, secs.*			
		Preliminary peak	*Peak value*	*Time to reach peak pr. t., hrs.*	*Time to return to normal, hrs.*
Rabbit					
11	100	20.0	20.3	18	42
16	100	18.6	24.4	27	45
18	50	18.9	32.7	24	...
19	50	45.6	72.5	27	...
20	50	21.8	29.6	24	30
11	25	....	21.4	24	42
17	25	21.2	28.5	24	42
Dog					
Sa	50	20.5	....	....	33
Sp	50	23.0	30.0	26½	36
P	50	19.2	17.5	26½	36
Rabbit	*dicoumarol*				
16	3	....	38.0	46	105
11	3	....	26.9	24	89
17	3	....	24.8	42	90
18	3	....	32.8	44	92
Dog					
Sa	50	....	43.0	66	92

Prothrombin time with 100% plasma and 0.004 M Ca. normal prothrombin times were 10 to 12 secs. for rabbits, 9 to 13 secs. for dogs.

x—Averages of three and two experiments.

single doses of the drug was studied. Preliminary experiments indicated that it was advisable to administer the drug every eight hours. Various amounts of the drug were given orally to dogs and rabbits every eight hours for periods of from 4 to 46 days. As shown in Fig. 2, repeated administration of P.I.D. resulted in a prolonged prothrombin time. Eight mgm./kg. every 8 hours resulted in a prolonged prothrombin time of over one minute. After 20 days, the drug was withdrawn and it can be seen that in 24 hours, the prothrombin time was normal. This appears to be the most valuable property of the drug. So rapid is the recovery that we have found that failure to ingest a single dose of P.I.D. is reflected in the prothrombin time. This may be responsible for irregularities observed in the figure. Essentially the same results were obtained whether the prothrombin times were determined with 0.004 M calcium or the more concentrated solutions usually used. Due to the insensitivity of the system for slight decreases in prothrombin, the action of P.I.D. is apparently delayed when the prothrombin time is measured with the latter.

Since an amount of drug which had little effect on prothrombin time when given in a single dose, had such a marked effect when the dose was repeated, the effect of number of injections in the twenty-four hours was studied (Fig. 3); 25 mgm./kg./24 hours was given in three divided doses for seven days, then as a single dose each twenty-four hours, and then

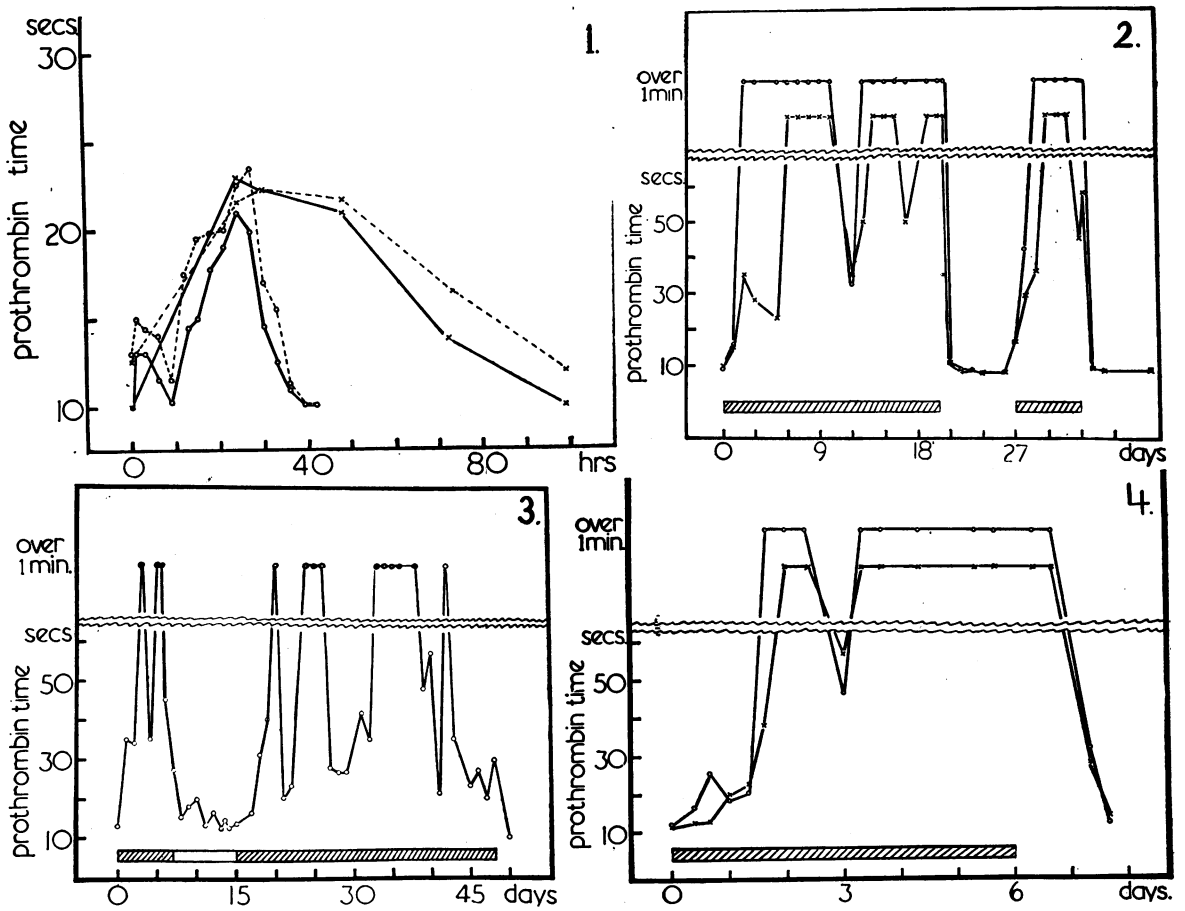

Fig. 1.—Effect of P.I.D. and dicoumarol on prothrombin time in the rabbit. Rabbit No. 11, 2 kg. x———x 6 mgm. dicoumarol 0———0 50 mgm. P.I.D. orally at zero time. Prothrombin time with 0.004 M Ca. Solid line shows values with undiluted plasma, broken line with 25% plasma. **Fig. 2.**—Effect of repeated doses of P.I.D. on prothrombin time of dogs. Dog Sp, 7.1 kg., 8 mgm./kg. given every eight hours. 0———0 prothrombin time with 0.004 M Ca., x———x with 0.02 M Ca. **Fig. 3.**—Comparison of effect of divided doses of P.I.D. on prothrombin time. ——— 25 mgm./kg./24 hours in single dose. ——— 25 mgm./kg./24 hours divided into three equal doses. Dog Pe, 6.8 kg. Ca. concentration, 0.004 M. **Fig. 4.**—Effect of P.I.D. and P.I.D. with vitamin K in rabbits. 50 mgm./kg. of P.I.D. and 50 mgm./kg. of vitamin K per day divided in three doses. x———x rabbit No. 16, P.I.D.; 0———0 rabbit No. 11, P.I.D. plus vitamin K.

finally in three divided doses for a further thirty days. The same rise in the prothrombin time to values above 1′ is observed. Replacing the dose of 8 mgm./kg. every 8 hours, by a dose of 25 mgm./kg. every 24 hours resulted in the prothrombin time falling almost to normal (ranging from 12 to 20 seconds). On the fourteenth day, the blood samples were taken every four hours to determine if there were marked variations in the prothrombin time during one day. Values during the course of the day did not show much variation, indicating that the failure to observe an increased prothrombin time with these single doses every twenty-four hours was not due to rapid changes in the prothrombin time occurring during the course of each twenty-four hour period.

While the dose levels used in Figs. 2 and 3 both give the same extremely prolonged prothrombin time when the drug was administered three times a day, maintenance of very prolonged prothrombin times was not always consistent, since marked fluctuations sometimes occurred in the value and it dropped occasionally for no apparent reason to the range of 20 to 30 seconds (two to three times normal). Several factors appeared to be contributory. One factor was failure on the part of the dog to ingest the drug, in spite of precautions taken to ensure this. Thus, on the morning of the fifth day, the capsule was found in the cage of dog Pe (Fig. 3) and it is suggestive that this failure to ingest the drug on the previous evening coincides with the low prothrombin values on the morning and evening of that day. This same factor may also be operative and explain some of the irregularities during the second period of eight hour dosage. As in this period, the blood samples were taken at much greater intervals, this gave the appearance of much longer periods of decreased prothrombin time and greater fluctuations (*e.g.*, for the twenty-third day). The fact that even missing one capsule per eight hour period affected the prothrombin time response indicates the rapid action of this drug compared to dicoumarol. Another factor operative may have been the protein level of the diet. Meat was fed to animals (Fig. 3) on the 24th to 30th days. It can be seen that the prothrombin time returned almost to normal. Foley and Wright[10] have already suggested that protein intake affects the effectiveness of dicoumarol.

In Fig. 4 is shown the prothrombin time response when P.I.D. was administered to rabbits (50 mgm./kg. was given daily in three divided doses). As with the dogs, a hypoprothrombinæmia was produced. The animals were maintained on the drug for six days. The prothrombin time reached the normal value forty-eight hours after the last dose of drug. The quantitative similarity in the response of the two species is remarkable. Some of the animals studied received simultaneously the same weight of vitamin K (2 methyl 1:4 naphthoquinone) as of P.I.D. and this was repeated at several dose levels of P.I.D. As shown in Fig. 4, there was no difference in response between the animal receiving P.I.D. and the one receiving P.I.D. and vitamin K. Hence, in contrast to results obtained in similar experiments with vitamin K and dicoumarol,[9] giving a weight of vitamin K equal to the weight of this prothrombopenic agent did not affect the change in prothrombin time in any way. Soulier and Gueguen reported that vitamin K had no effect on the increased prothrombin time produced by a single dose of P.I.D.

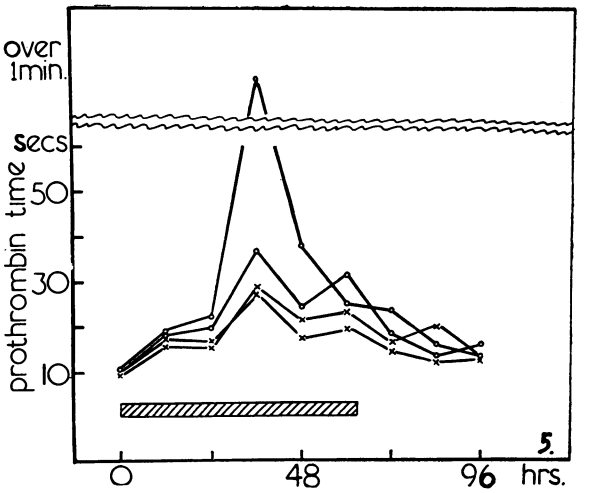

Fig. 5.—Effect of small doses of P.I.D. on prothrombin time. x ———— x 1 mgm./kg. every 8 hours. 0 ———— 0 2 mgm./kg. every 8 hours. Rabbits.

With repeated doses given at short intervals (8 hours), quite small doses of P.I.D. are effective in causing an increase in prothrombin time (Fig. 5). Doses of 2 mgm. and 1 mgm./kg. given every eight hours to rabbits resulted in the prothrombin time being increased to values between 20 and 40 seconds. One sample from one animal gave a prothrombin time of 165 seconds but this was not maintained. Variation during the maintenance period suggested that

some fluctuation in the prothrombin time occurred between dosage periods. With these low dosages, the same rapid rise in prothrombin time at the beginning of the experiment and the same rapid return to normal on stopping the drug was observed as with the high dosages. The effect of varying dosage has not been studied further, as it was considered of greater significance to determine the consistency of the response for the dosage levels selected. Repeated experiments with dogs and rabbits using the 8.3 and 16.7 mgm./kg. doses (25 and 50 mgm./kg./day) consistently gave the response shown in Figs. 2, 3 and 4. Since these dose levels give prothrombin times of infinity they represent the upper limit of dosage. Presumably there is a lower limit of dosage below which the drug fails to affect the prothrombin time. The values shown in Fig. 5 indicate that this level is approximately 1 mgm./kg. It appears that the prothrombin time response does not increase gradually with dosage but rather above an effective dose the response increases very rapidly. The results reported in Fig. 5 suggest that the administration of 3 mgm./kg./24 hours in divided doses maintains the prothrombin time satisfactorily at two or three times the normal value.

Toxicity of P.I.D.—All animals receiving the drug were studied for symptoms of toxicity. As with dicoumarol and any other drug which has a marked pharmacological action, one must distinguish the effects of overdosage of the drug (in this case the effects of a marked decrease in prothrombin) from side effects unrelated to the desired action of the drug and which constitute its true toxicity. A prothrombin time of infinity presumably indicates an undesirable degree of hypocoagulability of the blood and therefore represents overdosage. Hæmorrhage was a definite phenomenon with our animals when the prothrombin time became infinity. Hæmostasis could be satisfactorily produced by ordinary pressure if a 25 gauge needle was used for the blood sample. However, with an 18-gauge needle, hæmostasis was very difficult and prolonged bleeding from the veins tended to occur. There was no effect observed on the red cell, white cell or platelet count after continuous administration of P.I.D. No change was observed in the hæmatocrit value and differential white cell count, which were determined at intervals on some of the animals receiving the drug. Soulier and Gueguen reported renal damage with very large doses of the drug. Therefore, on all occasions when urine was collected, it was examined microscopically, with almost uniformly negative results. Two animals maintained on the drug for a long period of time with very prolonged prothrombin times showed interesting hæmorrhagic episodes. These were precipitated by extraneous factors (not related to P.I.D.) but were very severe. Stopping administration of the drug resulted in rapid and complete recovery. It was our impression, based on previous experience, that these animals would not have survived a similar condition following dicoumarol, without repeated transfusions and large doses of vitamin K.

Several dogs were maintained for fifty days on the drug, with infinite prothrombin times for most of the period, yet showed no hæmorrhagic condition at any time. Two rabbits and one of the dogs studied died during the investigation and a similar number were sacrificed. We are indebted to Dr. D. F. Moore for studying these animals for pathological lesions. No pathological lesions ascribable to the P.I.D. were observed histologically in heart, lungs, liver or intestine. In the kidney, a mild fatty degeneration particularly noticeable in the limbs of Henle's loops, was observed. These animals had received from 0.5 to 3.0 gm./kg. of P.I.D. over a period of some months. Possible long term toxicity of P.I.D. is still under investigation.

DISCUSSION

It is evident from the results reported that P.I.D. possesses valuable properties compared to dicoumarol in its ability to lengthen the prothrombin time, since cessation of the drug results in a prompt return of the prothrombin time to normal levels in approximately twenty-four to thirty hours in the animals studied. A single dose of P.I.D. has only a slight effect but if repeated doses at frequent intervals are administered, the prothrombin time can be increased to very high values. Hence, while with single doses, this drug appears to be a weak prothrombopenic agent compared to dicoumarol, on repeated administration it appears to be as effective as the latter. 3 mgm./kg./24 hours is suggested as a possible routine dose which compares closely with the clinical dose

of 300 mgm./24 hours for dicoumarol. While as with dicoumarol, overdosage with the drug results in a hæmorrhagic condition, cessation of the drug with the resultant return of the prothrombin time to normal completely reverses this serious condition, and hence there appears to be a much greater safety factor with this substance.

It is evident from our findings that the main toxic effects of P.I.D. as for dicoumarol, are associated with its effect on the clotting system of the blood and the resulting effects on the mechanisms of hæmostasis. These effects result when such large quantities of the drug are given that the prothrombin time is increased to infinity. Since clinically the drug will be controlled by determinations of the prothrombin time, such extreme overdosage will be avoided. The drug appears to be excreted by the kidneys, and hence, it is not surprising that it can cause renal damage, probably because of concentration and resulting precipitation of the drug in the tubules. However, Soulier and Gueguen and ourselves have observed indications of such renal damage only with extremely large overdosages of the drug in terms of its ability to maintain a prolonged prothrombin time.

In conclusion, the results of this study demonstrate that while P.I.D. is a poor prothrombopenic agent as judged by the ordinary test used for dicoumarol, it proves to be highly effective when the method of administration is changed. This suggests that the large series of compounds related to dicoumarol already tested for prothrombopenic action should be subjected to re-evaluation.

SUMMARY

1. The effect of 2-phenylindanedione-1, 3 (P.I.D.) on the prothrombin time of dogs and rabbits has been investigated. A decreased concentration of calcium was used in the estimation of prothrombin time to make it a more sensitive indicator of the action of prothrombopenic agents. The finding of Meunier, Mentzer, and Molho[1] that the prothrombin time returns to normal values more rapidly with this substance than after the administration of dicoumarol, has been confirmed.

2. While single doses of this compound had only a slight effect on prothrombin time, it was found that administration of P.I.D. at frequent intervals resulted in the drug being as active as dicoumarol in prolonging the prothrombin time. 8.3 mgm./kg. every 8 hours resulted in the modified prothrombin time becoming infinity after three days. 1 mgm./kg. every 8 hours increased the prothrombin time to approximately twice the normal value. Vitamin K had no effect on the prolonged prothrombin time produced. With withdrawal of the drug, the prothrombin time returned to normal within 36 hours.

3. Following large doses of the drug over a long period of time, a hæmorrhagic condition similar to that resulting from dicoumarol overdosage was observed when other precipitating factors were present. This was rapidly reversed after withdrawal of the drug. No gross toxic effects were observed with 8.3 mgm./kg., administered every eight hours for forty-eight days in dogs.

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A NEW PROTHROMBOPENIC AGENT*

Ancel Blaustein, M.D.

New York, N.Y.

ANTICOAGULANT therapy until recently has focussed attention on heparin and dicoumarol. These agents have been subjected to extensive experimental and clinical studies and their properties and actions are well established. Heparin has to be administered intravenously. It very rapidly prolongs the clotting time but its effect is of relatively short duration and consequently frequent administration is necessary.

The clotting time rather than the prothrombin time is utilized to follow the anticoagulant effect. Heparin appears to exert an anti-

* From the Department of Pathology, University of Vermont, College of Medicine, Burlington, Vt.