a differential urinary-folic-acid-excretion test (Girdwood, 1953b) will clarify the diagnosis. It should be noted that one cannot exclude malabsorption of folic acid merely by obtaining a normal result with a fat-balance test carried out over a short period of eight days.

Most of the cases reported here with megaloblastic anaemia in association with idiopathic steatorrhoea had a differential urinary-folic-acid-excretion test carried out, and there was evidence of malabsorption of folic acid and a normal level of serum vitamin B₁₂ in all cases except one. These findings explain why most patients with the "malabsorption syndrome" respond so much better to folic acid than to vitamin B₁₂.

In considering serum vitamin B₁₂ levels in pernicious anaemia patients under treatment, it is important to remember that in the normal person there are large stores of cobalamin substances, particularly in the liver (Girdwood, 1952), and that the serum measurement does not necessarily give any information about the extent of depletion of these stores. It is likely that megaloblastic anaemia in pernicious anaemia is a *late* manifestation of generalized deficiency of vitamin B₁₂. I have previously made the suggestion that subacute combined degeneration of the cord may develop in the absence of anaemia because of the presence of folic acid substances in the body, and shown (Girdwood, 1952) that substances with folic acid activity for the growth of micro-organisms may be present in the liver of a pernicious anaemia patient in the absence of vitamin B₁₂. The patient referred to above who had clinical features of subacute combined degeneration of the cord and a low serum vitamin B₁₂ level despite the presence of a normoblastic bone marrow may have had the megaloblastic changes but not the neurological complications prevented by the folic acid in the blunderbuss haematinic that had been given before she was referred to hospital.

Summary

The serum level of vitamin B₁₂ has been estimated by a *Lactobacillus leichmannii* growth method in 36 patients with pernicious anaemia in relapse, 24 patients with other forms of megaloblastic anaemia, 55 persons who did not suffer from megaloblastic anaemia, and 20 patients with pernicious anaemia under treatment.

The level was less than 130 $\mu\mu\text{g./ml.}$ in 34 out of 36 cases of pernicious anaemia in relapse, in one patient who had megaloblastic anaemia and another with macrocytic but not frankly megaloblastic anaemia after partial gastrectomy, in one with idiopathic steatorrhoea, and in three other persons, two of whom had macrocytic anaemia with a normoblastic marrow and who responded to vitamin B₁₂ therapy. It exceeded 130 $\mu\mu\text{g./ml.}$ in all the other patients tested.

The method is speedy, and together with the differential urinary-folic-acid-excretion test is of value in the investigation of the pathogenesis of complex cases of megaloblastic anaemia.

My thanks are due to my technician, Mr. Robert Brown, for carrying out many of the assays. The work was aided by the John Risk Bequest.

REFERENCES

- Girdwood, R. H. (1952). *Biochem. J.*, **52**, 58.
 — (1953a). *Blood*, **8**, 469.
 — (1953b). *Lancet*, **2**, 53.
 Hutner, S. H., Provasoli, L., Stokstad, E. L. R., Hoffmann, C. E., Belt, M., Franklin, A. L., and Jukes, T. H. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 118.
 Mollin, D. L., and Ross, G. I. M. (1953). *British Medical Journal*, **2**, 640.
 Rosenthal, H. L., and Sarett, H. P. (1952). *J. biol. Chem.*, **199**, 433.
 Ross, G. I. M. (1950). *Nature, Lond.*, **166**, 270.
 Thompson, H. T., Dietrich, L. S., and Elvehjem, C. A. (1950). *J. biol. Chem.*, **184**, 175.
 Unglaub, W. G., Rosenthal, H. L., and Goldsmith, G. A. (1954). *J. Lab. clin. Med.*, **43**, 143.

ANTAGONISTIC EFFECT OF ORAL VITAMIN K₁ ON THE ACTION OF ETHYL BISCOUMACETATE AND PHENYLINDANEDIONE

BY

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There are, to date, few British reports on the value of vitamin K₁ (2-methyl-3-phytyl-1:4:naphthaquinone) for antagonizing or modifying the "hypoprothrombinaemic" effect of the anticoagulant drugs dicoumarol (3,3'-methylene-bis-4-hydroxycoumarin), "tromexan" (ethyl biscoumacetate; bis-3:3'(4-hydroxycoumarinyl)-ethyl acetate), and "dindevan" (phenylindanedione; 2-phenyl-indane-1:3-dione). As the last two drugs are now widely used in the treatment of thrombo-embolic disease, the effect of fat-soluble vitamin K₁ in relation to the use of these anticoagulants requires emphasis.

Material and Methods

A series of experiments was performed on groups of healthy subjects who were taking regular doses of ethyl biscoumacetate or phenylindanedione in therapeutic amounts. At set times, while taking one or other of these

FIG. 1.—Prothrombin activities on stopping tromexan (ethyl biscoumacetate) administration. The arrow indicates when 100 mg. of vitamin K₁ was given orally.

drugs, a single oral dose of 100 mg. of vitamin K₁ was administered under supervision. The vitamin K₁ used was in the form of a solution with emulsifiers, which, when added to water, forms a finely dispersed emulsion having a particle size of about 3 μ . The solution was stored in brown glass bottles in a dark cupboard at room temperature. It was found convenient to administer the vitamin K₁ in a small amount of fruit juice in order to overcome its somewhat bitter taste. Mixing with the juice to form an emulsion was carried out immediately before a dose was given.

As a rule, experiments involving six individuals under the same regime were run in parallel. Prothrombin activity estimation was performed at frequent intervals under standard conditions. Venous blood was collected and 2 ml. amounts were measured into tubes containing 0.2 ml. M/10 potassium oxalate which had been evaporated to dryness. The tubes of blood were centrifuged, the plasma was separated, and estimations of prothrombin time were performed without delay, using a one-stage method (see Quick, 1951). Depending on the batch of human brain extract thromboplastin employed, normal prothrombin times ranged from 10 to 13 seconds. The same batch of thromboplastin giving a uniform normal value was used throughout each group of experiments. Calculation of so-called prothrombin activity was made by reference to an activity curve constructed by serial dilution of normal plasma with tricalcium-phosphate-absorbed normal plasma and the prothrombin time of such dilutions plotted on graph paper. *This method gives a straight-line graph with results which are often some 50% lower than activities recorded using a saline dilution curve in relation to blood samples obtained from patients receiving these anticoagulants.*

Patients under treatment with ethyl biscoumacetate or phenylindanedione who had low prothrombin activities were also given single doses of vitamin K₁ in amounts ranging from 10 to 100 mg., and similar laboratory studies were made.

Results

Fig. 1 shows the results of two separate experiments performed on each of six individuals. The continuous lines

show prothrombin activities during and after stopping administration of ethyl biscoumacetate. The broken lines show the values obtained in the same subjects some three weeks later when (on the fifth day of the experiments) a single oral dose of 100 mg. of vitamin K₁ was taken at the same time as the last dose of ethyl biscoumacetate. In each case the dose of vitamin K₁ significantly shortened the time taken for prothrombin activity to return to normal.

Fig. 2 shows the results of six experiments in which normal subjects were given 100 mg. of oral vitamin K₁ while maintained on therapeutic doses of ethyl biscoumacetate. In five instances, prothrombin activities were veering towards normal in four hours and showed normal activity between 24 and 30 hours after the vitamin K₁ had been taken. In experiment 6 the prothrombin activity was normal four hours after taking the dose of the vitamin. Values remained normal for one to three days even although ethyl biscoumacetate was continued in the same dosage. Prothrombin activities following the discontinuation of ethyl biscoumacetate are also shown.

Fig. 3 shows the influence of a single oral dose of 100 mg. of vitamin K₁ taken by six normal subjects while receiving phenylindanedione. All these subjects continued to take regular doses of phenylindanedione throughout the period of the trial, the dose of vitamin K₁ being given on the fourth or fifth day. In two subjects prothrombin activities were normal in 24 hours, and in three the values were normal when tested 48 hours after the dose of the vitamin had been taken. In the case of the remaining individual, the prothrombin activity was increased by some 40% within 48 hours.

Fig. 4 shows examples of the effect of 30-mg. doses of oral vitamin K₁ on the prothrombin activities of patients receiving treatment with ethyl biscoumacetate or phenylindanedione for thrombo-embolic disease. The modifying action of the vitamin on that of the anticoagulant is shown.

Reviewing results obtained from normal subjects and hospital patients and disregarding the length of time of anticoagulant administration, we have nine instances in which

FIG. 2.—The antagonistic effect to tromexan of a single dose of oral vitamin K₁. The arrow indicates administration of 100 mg. of vitamin K₁ orally.

FIG. 3.—The antagonistic effect of a single dose of oral vitamin K₁ to dicoumarol (phenylindanedione). The arrow indicates administration of 100 mg. of vitamin K₁ orally.

frequently repeated tests were performed on persons after ethyl biscoumacetate was stopped. The average time taken for the prothrombin activity to return spontaneously to normal was 44.3 hours ($\sigma=7.4$). In 18 instances, in which repeated testing was possible after the individuals had received 100 mg. of vitamin K₁ at the time of taking their last dose of ethyl biscoumacetate, the average time taken for the prothrombin activity to return to normal was 10.3 hours ($\sigma=4.8$). Employing frequent tests, it was found that six normal subjects, on stopping phenylindanedione, took an average time of 61.6 hours ($\sigma=10.9$) for the prothrombin activity to return to normal. In contrast, ten subjects similarly tested after taking 100 mg. of oral vitamin K₁ with their last dose of phenylindanedione showed an average time of 12.6 hours ($\sigma=6.3$) for their prothrombin activities to return to 100%.

Discussion

Early reports on the action of vitamin K₁ and its oxide in relation to dicoumarol therapy were made by Davidson and MacDonald (1943) and Lucia and Aggeler (1944). These and subsequent papers by James *et al.* (1948, 1949, 1951), Miller *et al.* (1950), and Douglas and Brown (1952) showed that, when given intravenously, vitamin K₁ and its oxide showed an antagonistic action to the dicoumarol group

of anticoagulants. Fat-soluble vitamin K₁ was much superior to water-soluble vitamin K analogues, which had a very limited or doubtful value in influencing hypoprothrombinaemia due to dicoumarol-like drugs.

The action of oral vitamin K₁ in influencing the effect of dicoumarol-like drugs or phenylindanedione has been reported by Reid (1951), Fuller and Barker (1951), and Toohey (1952, 1954). Koller (1952), using oral vitamin K₁ in the form of an emulsion, found that relatively very small doses were effective compared with those previously used—for example, by Toohey (1952), employing the simple oily substance. Koller found that an oral dose of the emulsion containing as little as 5 mg. could raise Quick's one-stage prothrombin time to normal, even although administration of a dicoumarol group drug was continued. In a recent report Toohey (1954), using the same emulsion as Koller and ourselves, found oral amounts of 5–15 mg. of vitamin K₁ sufficient to restore the prothrombin time to a safe therapeutic level in patients receiving short-acting anticoagulants. Other European reports on the effect of oral vitamin K₁ on the action of phenylindanedione have been made by Breda *et al.* (1952–3) and Perlick (1953).

Our experience, in common with that of most other authors using water-soluble vitamin K analogues (for example, Douglas and Brown, 1952), has been that such water-soluble preparations, given orally or parenterally in generous dosage, were either ineffective or of very doubtful value in influencing the one-stage prothrombin levels of patients receiving either dicoumarol-like drugs or phenylindanedione. While it has been shown that the coagulation defect caused by these drugs is probably due primarily to a reduction of factor VII, prothrombin itself is also affected. However, the term "hypoprothrombinaemia" is, in general, used in this respect to cover a reduction of both these factors and possibly other components of the coagulation mechanism.

The practical importance of the vitamin K₁ emulsion which is now generally available lies in giving the physician a substance which can rapidly modify an unduly low or potentially dangerous drug-induced hypoprothrombinaemia or correct it in the emergency of haemorrhage.

For the experiments recorded in this paper, 100-mg. doses of oral vitamin K₁ in special emulsion were used throughout. This is obviously far too large an amount to use as a modifying dose in practice, as it would carry with it the risk of making the patient temporarily resistant to the anticoagulant drug for some days as well as that of running the danger of further thrombosis occurring after a rapid return of the prothrombin level to normal. As in our experience doses of 30 mg. of oral vitamin K₁ have been shown to raise the prothrombin activity by about 20% in 6 to 12 hours while anticoagulant therapy is maintained, it is now our practice to use oral doses of 10–15 mg. of oral vitamin K₁ in the emulsion form to bring unduly low prothrombin activities to our desired therapeutic level of 5–15%. These single doses of vitamin K₁ are particularly valuable when so-called loading doses of ethyl biscoumacetate or phenylindanedione have caused too great a depression of prothrombin activity in patients who are unduly sensitive to the action of these anticoagulants.

The availability of vitamin K₁ should not be regarded as making strict supervision of all patients under treatment with these anticoagulants any less essential, but the knowledge of its powerful antagonistic action will increase confidence in using ethyl biscoumacetate and phenylindanedione in amounts sufficient to reduce prothrombin activity to those low levels which are regarded by most workers as therapeutically desirable.

Summary

A series of experiments is described in which normal subjects were given oral doses of emulsified vitamin K₁, while receiving the anticoagulant drugs ethyl biscoumacetate or phenylindanedione.

FIG. 4.—Influence of 30-mg. doses of oral vitamin K₁ on prothrombin levels of patients taking tromexan or dicoumarol.

A marked antagonistic effect of the vitamin K₁ on the hypoprothrombinaemia caused by these drugs is shown.

Patients with low prothrombin activities, when given vitamin K₁ emulsion, showed modification of the prothrombin level while maintained on regular dosage of either ethyl biscoumacetate or phenylindanedione.

The application of these effects to the management of anticoagulant therapy is discussed. It is emphasized that small doses of vitamin K₁ of the order of 10–15 mg., when used in this particular form, should normally be employed if states which are refractory to the action of these anticoagulant drugs are to be avoided.

The fact that vitamin K₁ is now generally available makes it safer to use dicoumarol-group drugs or phenylindanedione in obtaining therapeutic levels of hypoprothrombinaemia.

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REFERENCES

- Breda, R., Ferrari, A., and Sideri, G. (1952-3). *Atti Soc. lombarda Sci. med. biol.*, **8**, 88.
 Davidson, C. S., and MacDonald, H. (1943). *New Engl. J. Med.*, **229**, 353.
 Douglas, A. S., and Brown, A. (1952). *British Medical Journal*, **1**, 412.
 Fuller, B. F., and Barker, N. W. (1951). *Minn. Med.*, **34**, 326.
 James, D. F., Bennett, I. L., jun., Scheinberg, P., and Butler, J. J. (1948). *Amer. J. Med.*, **4**, 455.
 — (1949). *Arch. intern. Med.*, **83**, 632.
 Johnson, T. D., and Burnett, S. (1951). *Amer. J. Med.*, **11**, 244.
 Koller, F. (1952). *Méd. et Hyg., Genève*, **10**, 339.
 Lucia, S. P., and Aggeler, P. M. (1944). *Proc. Soc. exp. Biol., N.Y.*, **56**, 36.
 Miller, R., Harvey, W. P., and Finch, C. A. (1950). *New Engl. J. Med.*, **242**, 211.
 Perlick, E. (1953). *Klin. Wschr.*, **31**, 261.
 Quick, A. J. (1951). *The Physiology and Pathology of Hemostasis*. Kimpton, London.
 Reid, R. A. (1951). *Quart. Bull. Northw. Univ. med. Sch.*, **25**, 292.
 Toohey, M. (1952). *British Medical Journal*, **2**, 687.
 — (1954). *Ibid.*, **1**, 1020.

TRIAL OF HEXYLRESORCINOL AS AIR DISINFECTANT FOR PREVENTION OF COLDS IN OFFICE WORKERS

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Hexylresorcinol was recommended for use as an air disinfectant by Twort and Baker (1942). Bourdillon *et al.* (1948), in their comparative study of a variety of possible disinfectant agents, showed that as little as 2 µg. per cu. ft. (about 70 µg. per m.³) was needed to produce a killing rate of 10 an hour against natural salivary bacteria sprayed from the mouth; but they could not obtain a killing rate of 30 an hour with any attainable concentration. The only field investigation reported appears to be that of Dickson (1953), who dispersed hexylresorcinol from "aerovap" vaporizers in a number of government offices in Dublin, and recorded a decrease in the amount of sick-absence among the staff in the treated rooms.

During the winter of 1951–2 we made some preliminary observations on the incidence of colds among the staff of the central offices of the then Ministry of National In-

surance in Newcastle-upon-Tyne, using the personal-interview method of enumerating colds described by Reid, Williams, and Hirsch (1953). Since the attack rates recorded in six separate rooms, each with about 45 staff, were very similar, the situation seemed suitable for a test of the value of hexylresorcinol for the reduction of the incidence of colds. This paper reports our failure to achieve any reduction in a carefully controlled trial carried out in the winter of 1952–3.

Material and Methods

Population Studied.—The investigation was made in eight rooms, each of about 26,000 cu. ft. (736 m.³) capacity, and each having 40–45 staff. They were particularly suitable for a controlled study, since the rooms were identical in size and shape, and very similar in the layout of furniture and fittings. The staff in each room were very similarly distributed in respect of age, sex, and status. In two groups of five and three rooms, the work done was identical. The observations made during 1951–2 had shown that the attack rates for colds in each of six rooms (similar to and including some of those observed in the present work) did not differ greatly, the room attack rates varying from 4.5 to 6.2 per 100 person-weeks of exposure.

Recording of Illness.—As in the study reported by Reid *et al.* (1953), a nurse visited each member of the staff once a week and inquired for a history of any upper respiratory illness occurring during the past week. The great majority of the incidents recorded were typical common colds, with no symptoms of fever and no aches or pains. The symptoms for each incident were recorded, and we can distinguish a group of influenza-like illnesses; there was, however, no epidemic of virus influenza recognized in Newcastle during the winter of 1952–3. For the purpose of analysis we have not regarded any cold as a new cold if it occurred in the week immediately succeeding a week in which another new cold was recorded.

Installation of Vaporizers.—Four "aerovap" vaporizers, each designed to yield 30 mg. of hexylresorcinol an hour, were installed in each of six rooms. These vaporizers were simple electrically heated pots which maintain the hexylresorcinol at a temperature of about 116° C. Distribution of the vapour around the room was dependent on natural air currents. During the period September 8, 1952, to January 22, 1953, hexylresorcinol was put into the vaporizers in three of the rooms, A, B, and C. Three other rooms, D, E, and F, had vaporizers running but containing only an inert material. Two further rooms, G and H, had no vaporizers. On January 22 the hexylresorcinol was transferred to the vaporizers in rooms D, E, and F without informing either the occupants of the rooms or the nurse who recorded the colds. Rooms A, B, and C then had the inert material.

Relative Humidity.—The relative humidity in the offices was recorded twice weekly; it varied between 50 and 60% throughout the investigation except for a short period in March and April, 1953, when it fell to 40–50%.

Air Sampling.—On four occasions during the winter, samples were collected from the air for bacteriological analysis with a slit-sampler modified from those described by Bourdillon *et al.* (1948). Two different counts of airborne bacteria are recorded in the tables. The "general count" is the number of particles carrying viable bacteria that grow on serum agar incubated at 37° C. for 18–20 hours. The count of "mouth streptococci" was obtained by method C of Williams and Hirsch (1950), and is an estimate of the number of streptococci thought to be derived directly or indirectly from the mouths of the occupants of the room. The *Str. salivarius* count is another measure of mouth pollution of the air. Air samples for estimation of hexylresorcinol content were taken on one occasion, the air being bubbled through phosphate buffer, which was subsequently reacted with diazotized sulphanilic acid.