been postulated as the basis of the acid-fast character of these organisms. There is little doubt that granules consisting predominantly of proteins, lipids, or polysaccharides occur irregularly in many organisms and in different metabolic states of the same organism.

These considerations naturally provoke speculation about the status of the nuclear bodies stained by Stille (1937), Piekarski (1937, 1939) and Robinow (1939, 1942) in numerous bacteria and which have come to occupy a somewhat controversial position in bacteriology in recent years. It is quite clear that electron microscopy does not settle the issue, since many organisms which show such bodies when stained by the Feulgen or Giemsa technique are too opaque for study with electrons. On the other hand, it is possible that some of the stained organisms are secondary forms, and what is taken to be a nucleus is in fact the shrunken body of the organism, especially since the cytoplasm of most bacteria is diffusely basophilic. In the few cases (Piekarski and Ruska, 1939; Robinow and Cosslett, 1948) where the same organism has been stained and examined in the electron micrograph a somewhat surprising result has been obtained—namely, where the nuclear body occurs in the stained specimen a lighter vacuole-like area appears in the electron microscope. This suggests that the true nuclear inclusion is more hydrated than other inclusions, and more so even than the surrounding cytoplasm. While such a state of affairs is contrary to general cytological experience, it is perhaps not surprising to find it in organisms as dense as are most bacteria.

Observations have as yet been too few to allow generalizations to be made regarding the morphology of bacteria. It is clear that the greatest single difficulty in the way of progress lies in the variability of organisms of the same culture.

REFERENCES

Anderson, T. F. (1943). *J. Bact.*, **45**, 303.
Boswell, F. W. (1947). *Brit. J. exp. Path.*, **28**, 253.
Brieger, E. M., Crowe, G. R., and Cosslett, V. E. (1947). *Nature*, **160**, 864.
Chu, C. M., Dawson, I. M., and Elford, W. J. (1949). *Lancet*, **1**, 602.
Cooper, P. D., Rowley, D., and Dawson, I. M. (1949). *Nature*, **164**, 842.
Cosslett, V. E. (1947). *J. appl. Physics*, **18**, 844.
—— (1948). *Biochim. Biophys. Acta, Amst.*, **2**, 239.
Cunha, R., Weil, M. L., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W. (1947). *J. Immunol.*, **55**, 69.
Dawson, I. M., and Elford, W. J. (1949). *J. gen. Microbiol.*, **3**, 298.
—— and McFarlane, A. S. (1948). *Nature*, **161**, 464.
Elford, W. J., Chu, C. M., Dawson, I. M., Dudgeon, J. A., Fulton, F., and Smiles, J. (1948). *Brit. J. exp. Path.*, **29**, 590.
Eyer, H., and Ruska, H. (1944). *Z. Hyg. InfectKr.*, **125**, 483.
Green, R. H., Anderson, T. F., and Smadel, J. E. (1942). *J. exp. Med.*, **75**, 651.
Hamre, D., Rake, H., and Rake, G. (1947). Ibid., **86**, 1.
Heinmets, F. (1948). *J. Bact.*, **55**, 823.
Hillier, J. (1946). *J. appl. Physics*, **17**, 307.
Jungeblut, C. W., and Bourdillon, J. (1943). *J. Amer. med. Ass.*, **123**, 399.
Kottmann, U. (1942). *Arch. ges. Virusforsch.*, **2**, 388.
Kurotchkin, T. J., Libby, R. L., Gagnon, E., and Cox, H. R. (1947). *J. Immunol.*, **55**, 283.
Lembke, A., and Ruska, H. (1940). *Klin. Wschr.*, **19**, 217.
Mandle, R. J. (1947). *Proc. Soc. exp. Biol., N.Y.*, **64**, 362.
Markham, R., Smith, K. M., and Wyckoff, R. W. G. (1948). *Nature*, **161**, 760.
Marton, L., Das Gupta, N. N., and Marton, C. (1946). *Science*, **104**, 35.
Melnick, J. L. (1944). *J. Immunol.*, **48**, 25.
Mosley, V. M., and Wyckoff, R. W. G. (1946). *Nature*, **157**, 263.
Mudd, S., Heinmets, F., and Anderson, T. F. (1943). *J. exp. Med.*, **78**, 327.
—— Polevitzky, K., and Anderson, T. F. (1942). *Arch. Path.*, **34**, 199.
Nagler, F. P. O., and Rake, G. (1948). *J. Bact.*, **55**, 45.
Perutz, M. F. (1948). *Nature*, **161**, 204.
Piekarski, G. (1937). *Arch. Mikrobiol.*, **8**, 428.
—— (1939). *Zbl. Bakt.*, **144**, Beiheft, 140.
—— and Ruska, H. (1939). *Arch. Mikrobiol.*, **10**, 302.
Plotz, H., Smadel, J. E., Anderson, T. F., and Chambers, L. A. (1943). *J. exp. Med.*, **77**, 355.
Rake, G., Rake, H., Hamre, D., and Groupé, V. (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 489.
Robinow, C. F. (1942). *Proc. roy. Soc. B.*, **130**, 299.
—— (1944). *J. Hyg., Camb.*, **43**, 413.
—— and Cosslett, V. E. (1948). *J. appl. Physics*, **19**, 124.
Ruska, H. (1941). *Z. Hyg. InfectKr.*, **123**, 294.
—— (1942). *Arch. ges. Virusforsch.*, **2**, 345.
—— (1943). *Ergebn. Hyg. Bakt.*, **25**, 437.
—— and Kausche, G. A. (1943). *Zbl. Bact.*, **150**, 311.
Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W. (1943). *Arch. Path.*, **36**, 167.
Sigurgeirsson, T., and Stanley, W. M. (1947). *Phytopathology*, **37**, 26.
Stille, B. (1937). *Arch. Mikrobiol.*, **8**, 125.
Teitel-Barnard, A. (1932). *Arch. roum. Path.*, **5**, 389.
Tiselius, A., and Gard, S. (1942). *Naturwissenschaften*, **30**, 728.
Tschudin, K. (1946). *Helv. phys. Acta*, **19**, 91.
Van Iterson, W. (1947). *Biochim. Biophys. Acta. Amst.*, **1**, 527.
Von Ardenne, M. (1948). *Kolloidzschr.*, **111**, 22.
Watson, J. H. L. (1947). *J. appl. Physics*, **18**, 153.
Weiss, L. J. (1943). *J. Immunol.*, **47**, 353.
Williams, R. C., and Wyckoff, R. W. G. (1946). *J. appl. Physics*, **17**, 23.
Wyckoff, R. W. G. (1946). *Science*, **104**, 36.
—— (1948a). *Nature*, **162**, 649.
—— (1948b). *Biochim. Biophys. Acta, Amst.*, **2**, 27.
—— (1948c). Ibid., **2**, 246.
—— (1948d). *J. appl. Physics*, **19**, 125.

CLINICAL TESTS OF A NEW COUMARIN SUBSTANCE

A REPORT TO THE MEDICAL RESEARCH COUNCIL

BY

CATHERINE C. BURT, B.Sc., M.B., Ch.B.

HELEN PAYLING WRIGHT, Ph.D., L.M.S.S.A.

AND

MIRKO KUBIK, M.D.Prague

(From the Department of Surgery, Edinburgh University, and the Obstetric Unit, University College Hospital, London)

This paper records the results of clinical tests of a new coumarin product, bis-3,3'-(4-oxycoumarinyl) ethyl acetate* (referred to here as B.O.E.A.), which seems to be a step nearer the ideal coumarin substance than any so far available for clinical use. Rosicky (1944) assumed that the delay in action of dicoumarol was due to difficulty in splitting the molecule, a biological fact later confirmed experimentally by Pulver and von Kaulla (1948). Rosicky therefore weakened the methylene linkage between the two coumarin groupings by the addition of a carboxyl group. Chemically the substance so produced was the ethyl ester of di-4-oxycoumarinyl acetic acid. Weight for weight this substance (B.O.E.A.) is about four times less active than dicoumarol — 3,3'-methylene-bis-(4-hydroxycoumarin) — 100 mg. of the latter corresponding approximately in anticoagulant action with 400 mg. of the former (Reinis and Kubik, 1948). B.O.E.A. is therefore prepared for clinical use in tablets of 0.3 g. for oral administration.

The comparative rates of action and excretion are set out in Fig. 1, which shows the average effect of a single dose of B.O.E.A. and of dicoumarol on the prothrombin levels in six subjects. In this group, after a single dose of B.O.E.A. the minimal prothrombin level was reached between eight and 24 hours and equally rapidly returned towards normal. With dicoumarol the minimal level was not reached for 32 hours and was maintained for a

*We wish to acknowledge the courtesy of SPOFA (United Pharmaceutical Works), Prague, and of Pharmaceutical Laboratories, Geigy Ltd., Manchester, who supplied this substance for trial under the respective names of "pelentan" and "tromexan."

comparatively long period. The rapidity of action and excretion of B.O.E.A. as compared with dicoumarol was reported in animal experiments by von Kaulla and Pulver (1948).

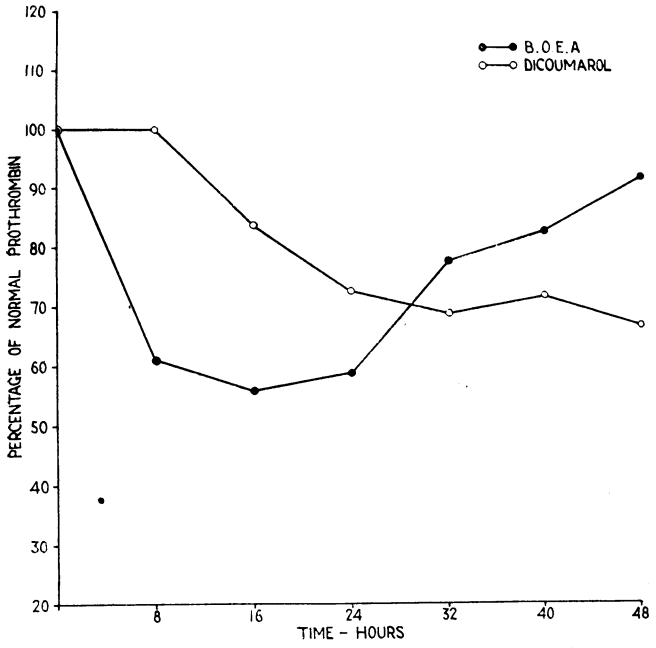

FIG. 1.—Average prothrombin percentage of normal in six subjects at eight-hourly intervals after (1) 1.2 g. of B.O.E.A., and (2) 300 mg. of dicoumarol given at 0 hours in each case.

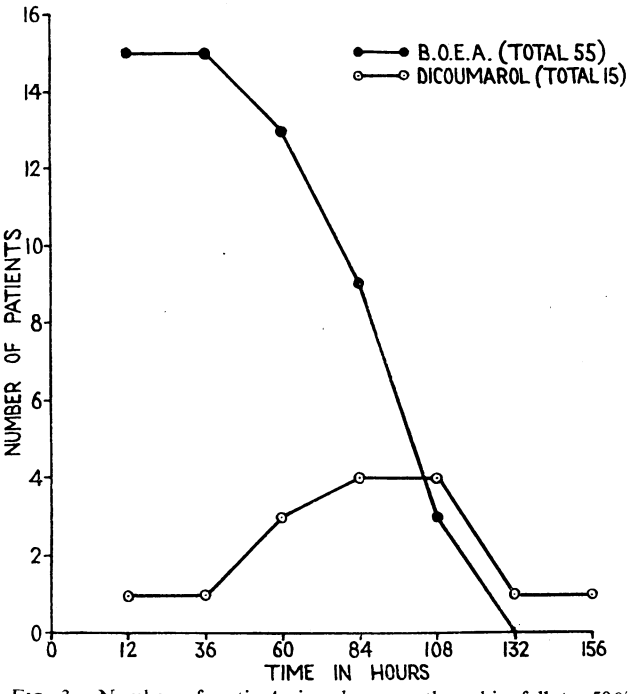

FIG. 3.—Number of patients in whom prothrombin fell to 50% or under of normal level, plotted against time after administration of B.O.E.A. in 55 cases, and comparable doses of dicoumarol in 15 cases (300 mg. followed by 200 mg. with maintenance of 100–200 mg. daily). Comparison between numbers at the required prothrombin level at 36 hours or under with those over 36 hours gives $\chi^2 = 6.3$ and P less than 0.02, which is highly significant.

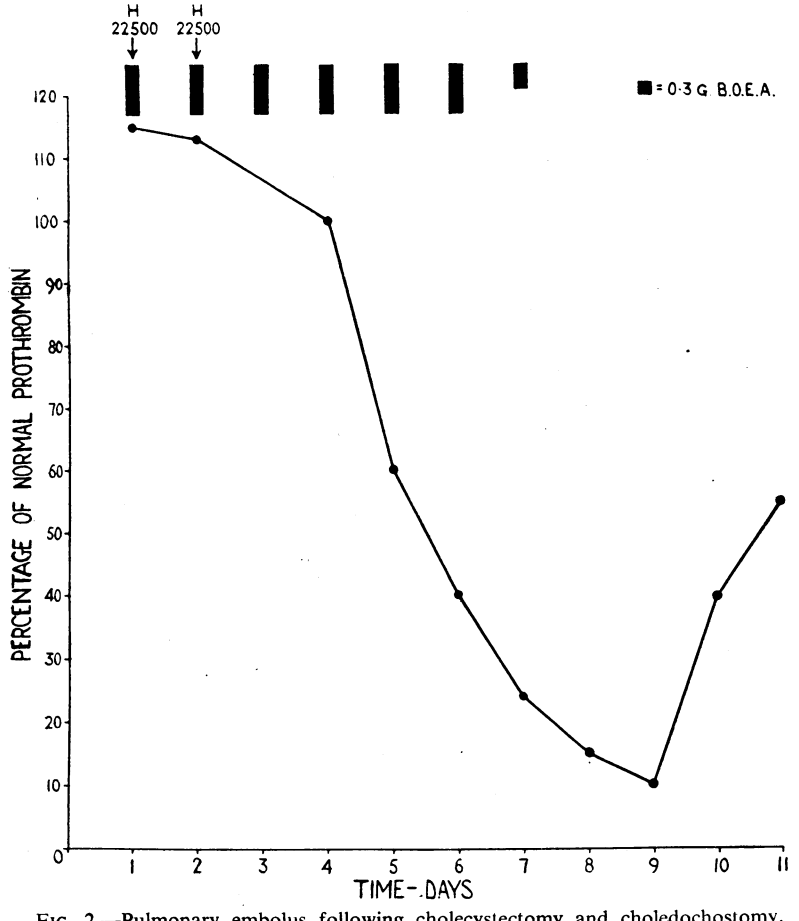

FIG. 2.—Pulmonary embolus following cholecystectomy and choledochostomy. Resistant to B.O.E.A. Effect of B.O.E.A. prolonged for two days after withdrawal. Good clinical recovery.

In the present trial, 126 subjects have been treated therapeutically with B.O.E.A. The cases include post-operative thrombosis and pulmonary embolism in patients in surgical and gynaecological wards, spontaneous venous thrombosis, puerperal thrombosis, and a group of patients with arterial thrombosis or embolism (Table I). In the surgical post-operative group many of the patients were gravely ill, apart from the thrombotic complication, while two patients in this group and four with arterial emboli also had auricular fibrillation.

Dosage and Duration of Treatment

In the majority of cases 0.9 to 1.2 g. of B.O.E.A. was given on the first two days, and thereafter dosage was regulated by response to treatment as shown by the prothrombin level and clinical signs, 0.3 to 0.6 g. usually proving sufficient. It is generally desirable to maintain the prothrombin level between 20 and 30% of normal, but in some cases a level of 40–50% of normal proved satisfactory for clinical improvement. The prothrombin level of the blood was usually checked daily, a procedure which is strongly advocated as a guard against overdosage (Allen, 1947) in all cases under treatment with dicoumarol. In our experience, once the desired prothrombin level is reached it has been maintained so steadily on 0.3 to 0.6 g. of B.O.E.A. daily that estimations need be made only on alternate days. In cases in which an immediate response to anticoagulant therapy was necessary heparin was given in addition for the first 24–48 hours,

and in a few cases dicoumarol was substituted for B.O.E.A. when supplies of the latter became short (Table II).

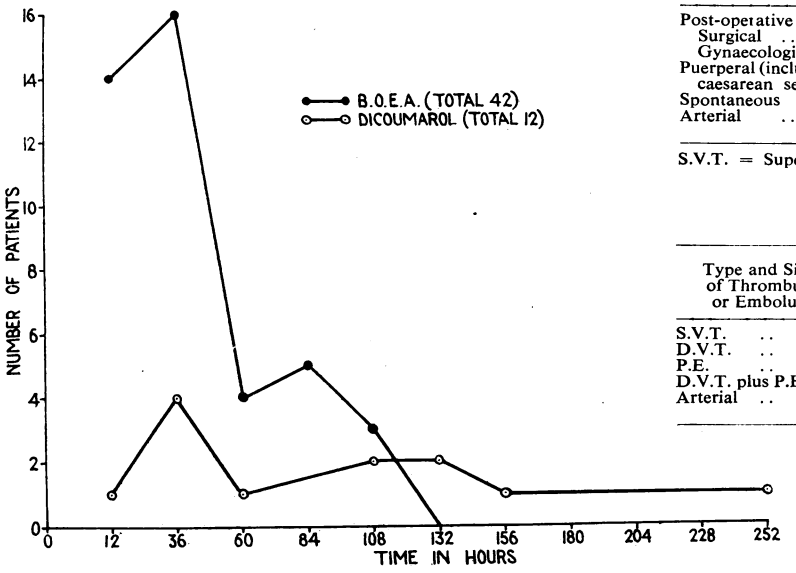

FIG. 4.—Number of patients in whom prothrombin rose to 50% or over of the normal level, plotted against time after the withdrawal of the drug. (A total of 54 patients, 42 of whom were treated with B.O.E.A. and 12 with dicoumarol.) Comparison between numbers at the required prothrombin level at 36 hours or under with those over 36 hours gives $\chi^2 = 3.6$ and P between 0.1 and 0.5, which is barely significant.

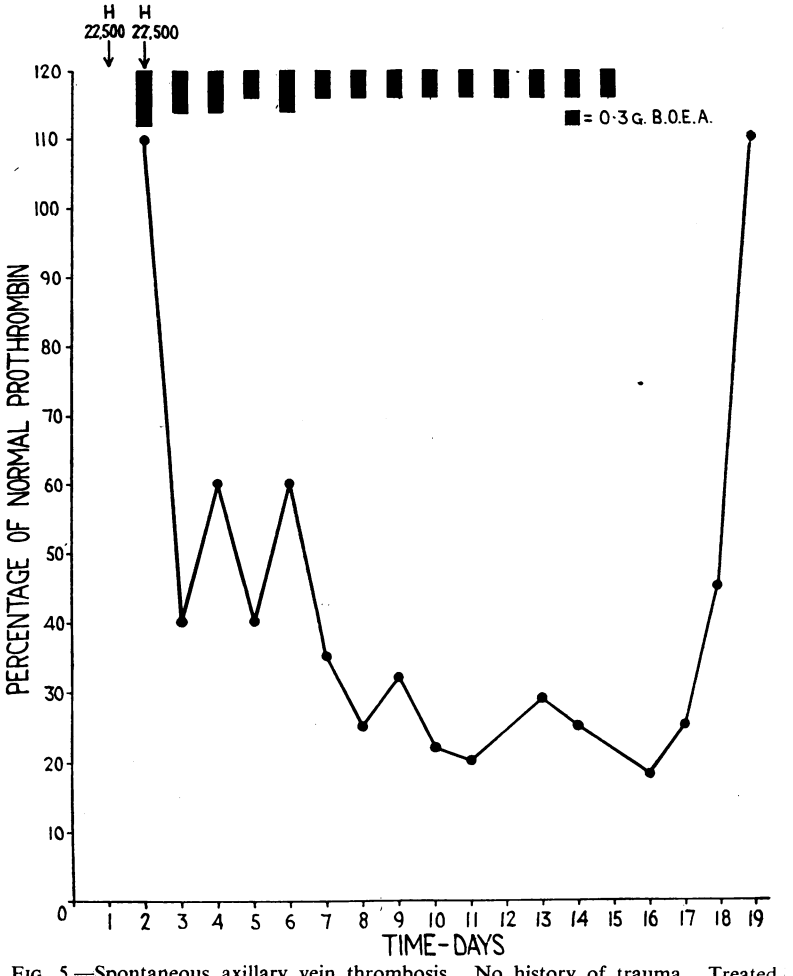

FIG. 5.—Spontaneous axillary vein thrombosis. No history of trauma. Treated with elevation, anticoagulants, and massage, with good results.

TABLE I.—*Subjects Treated with B.O.E.A.*

Type of Thrombosis	Average Age	No. of Cases	S.V.T.	D.V.T.	P.E.	D.V.T. plus P.E.
Post-operative:						
Surgical	58	37	1	30	3	3
Gynaecological ..	54	35	17	10	6	3
Puerperal (including 4 caesarean sections)	28	32	22	7	2	1
Spontaneous ..	39	9	—	9	—	—
Arterial	55	13	—	—	—	—

S.V.T. = Superficial venous thrombosis. D.V.T. = Deep venous thrombosis P.E. = Pulmonary embolus.

TABLE II.—*Method of Treatment*

Type and Site of Thrombus or Embolus	Total No.	B.O.E.A.	B.O.E.A. plus Heparin	B.O.E.A. plus Heparin plus Dicoumarol	B.O.E.A. plus Dicoumarol
S.V.T.	40	39	—	1	—
D.V.T.	56	23	30	1	2
P.E.	11	—	10	1	—
D.V.T. plus P.E. ..	7	—	6	1	—
Arterial	13	5	8	—	—

The majority of patients were under treatment for five to 14 days (average 11 days in the surgical group). In four patients with iliofemoral thrombosis treatment was continued for three to four weeks, and two patients with gangrene of the toes, due to arterial thrombosis, were each treated for two months. In one male patient with severe widespread recurrent venous thrombosis (who is still under treatment) the prothrombin level has been maintained in the region of 20 to 40% of normal for 10 months, the rather larger dosage of 0.6 to 0.9 g. having been necessary in this particular case to ensure the desired level.

Effect on Prothrombin Level

In over 80% of the patients under treatment the prothrombin level of the blood dropped to 50% or under within 36 hours of administration of the first dose of B.O.E.A., and it returned to the same level within the same period after discontinuance of the drug. Twenty subjects were regarded as resistant in that the prothrombin level failed to fall to 50% or under within 60 hours of the first dose. In 15 cases a lag of more than 60 hours in the return to 50% (or over) of the normal prothrombin level occurred at the end of treatment. A typical case is shown in Fig. 2.

Of one group of 70 patients suffering from major thrombotic or embolic episodes 55 were treated with B.O.E.A. and 15 with dicoumarol (initial dose 300 mg., and 200 mg. on the second day). A more rapid fall in prothrombin level occurred in the group treated with B.O.E.A. (Fig. 3). Although the number treated with dicoumarol is small, Yates's modification of the χ^2 test (Fisher, 1948) applied to the numbers responding or failing to respond within 36 hours of administration of the drug indicates that the difference is significant—a value for P of between 0.02 and 0.01 being obtained. Analysis of figures on the rate of elimination of the drug after cessation of treatment is shown in the same manner in Fig. 4.

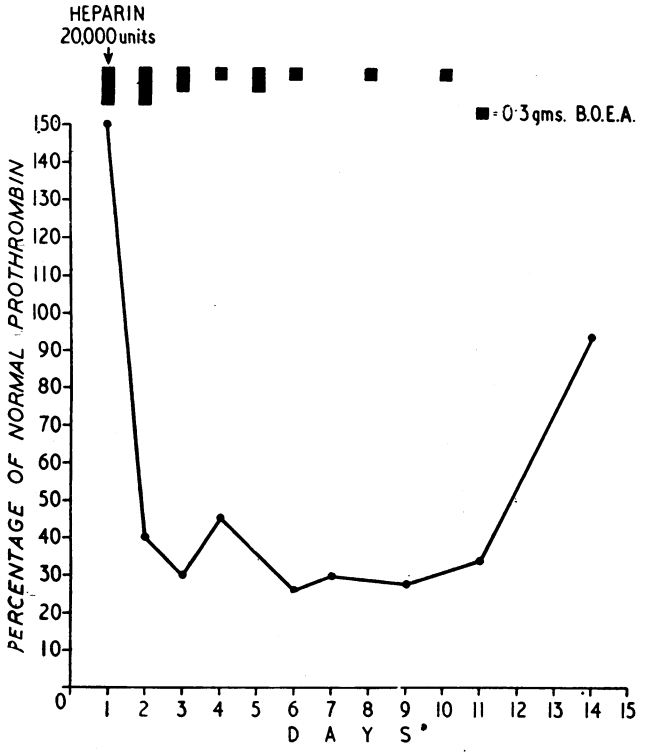

FIG. 6.—Normal response to B.O.E.A. Thrombosis of right femoral vein in patient with mitral stenosis on second day after normal delivery. Patient was ambulatory six days after starting treatment, and was discharged one week later.

A slow initial response to the drug was not invariably followed by a prolonged effect. On the contrary, seven of the patients in whom the prothrombin level had not returned to 50% within 60 hours had responded to the initial dose within 36 hours.

Clinical Results

The clinical results were good in all cases of post-operative venous thrombosis; residual swelling has persisted in only three cases. Since lack of space makes it impossible to give the detailed case histories, Fig. 5 is included to show a typical response. Good recovery occurred in the group of patients with pulmonary emboli, with the exception of one who died during treatment with B.O.E.A. This patient had a history of chronic bronchitis and bronchiectasis, and was admitted to a medical ward critically ill with an ilio-femoral thrombosis followed by a pulmonary embolus; she was also found to have auricular fibrillation. She responded well initially to heparin and B.O.E.A., and her prothrombin level was maintained between 20 and 40% of normal from the fourth to eleventh days. On the eleventh day, when the prothrombin level was 33% of normal, she became distressed, haemoptysis occurred, and her pulse rate rose steadily until she died on the morning of the twelfth day of treatment. Necropsy revealed a large old embolus in the pulmonary artery, not causing complete blockage, so that the lung showed no signs of infarction; spreading thrombosis had not occurred. Death was due to myocardial failure.

In the group of patients with obliterative arterial disease the aim of treatment was to prevent consecutive thrombosis and consequent development of or increase in gangrene. This was achieved in all except one case—that of a man with aortic thrombosis who died two days after operation.

In several cases in the present series the drug was administered within six hours of operation or delivery (Fig. 6). No excess puerperal or post-operative haemorrhage has been observed in cases treated for varying periods following operation or delivery. No infant suckled by a mother on B.O.E.A. has shown the tendency to bleed reported by Barnes and Ervin (1946), caused by the ingestion of anticoagulant secreted in the breast milk.

Toxic or Other Side-effects

No general toxic effects have been observed. A small number of patients complained of the bitter taste of the tablets, which persists for a considerable time; it probably accounted for the nausea initially complained of by three patients who vomited some of the tablets.

Haematuria occurred in one patient who was in error given the ordered dose twice on the fourth day of treatment (Fig. 7). The mistake was not recognized until almost 24 hours later, when 50 mg. of vitamin K was given. Red blood cells were found in the urine 24 hours after the second dose; haematuria was marked in the next specimen, and then gradually diminished during the following 12 hours. Another haemorrhagic incident occurred in a female patient with a grossly swollen leg due to an

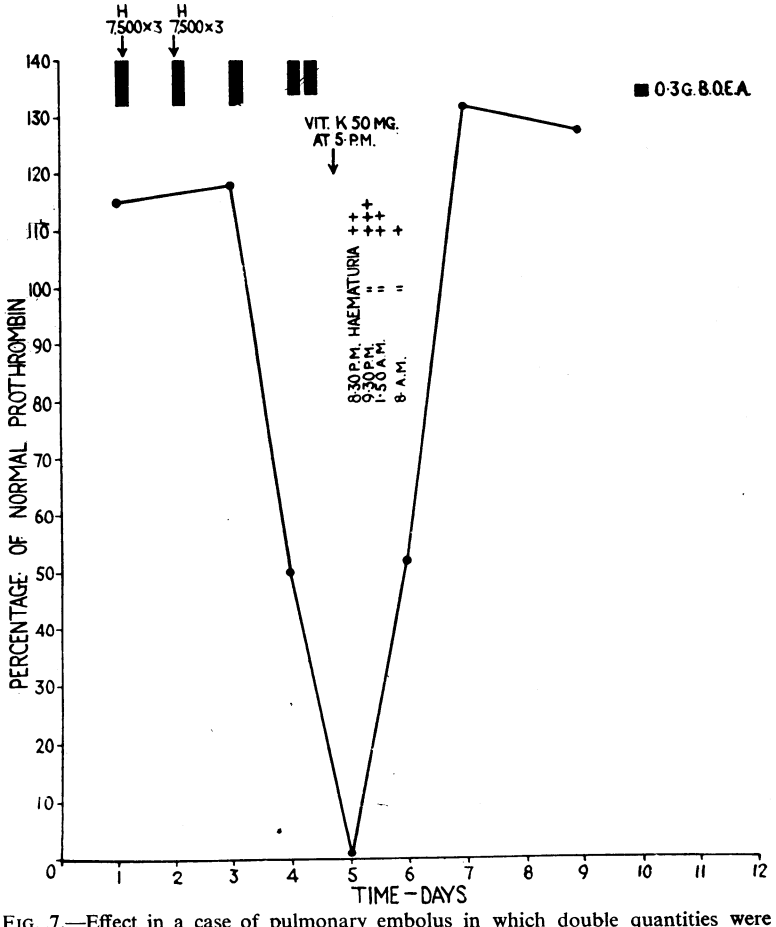

FIG. 7.—Effect in a case of pulmonary embolus in which double quantities were given in error.

ilio-femoral thrombosis. On the fifth day of treatment, when the prothrombin level was 28% of normal, numerous petechial haemorrhages appeared along the line of the saphenous vein and its tributaries (a septic thrombosis had previously been present in this vein following an intravenous drip). On the sixth day these petechial haemorrhages had spread round the leg and thigh, and large blood blisters were present around the drip scar and on the posterior surface of the thigh. The capillary resistance, however, was within normal limits in both the normal and the affected foot. Possibly sepsis and, in the thigh, pressure from a bed-pan may have been the causal factors. No further haemorrhage occurred when anticoagulant therapy was discontinued.

Discussion

We feel that bis-3,3′-(4-oxycoumarinyl) ethyl acetate is certainly a step forward towards the production of an ideal anticoagulant, but it is still necessary to take the precautions advocated for this type of therapy. Prothrombin estimations should be carried out daily, in a reliable laboratory by a recognized technique, before the desired level is reached, and at least every other day while the patient is under treatment. If this precaution is not rigorously adhered to there is a danger of haemorrhage, though this is unlikely to be severe, since the elimination of B.O.E.A. is more rapid than with dicoumarol. The case illustrated in Fig. 7 shows, however, that overdosage may cause a haemorrhagic episode.

Summary

Clinical results in 126 patients with venous thrombosis, pulmonary emboli, arterial thrombosis, or emboli who have been treated with a new coumarin compound, bis-3,3′-(4-oxycoumarinyl) ethyl acetate for periods varying from five days to ten months are reported.

In over 80% of cases given adequate dosage the prothrombin level of the blood was reduced to under 50% of normal within 36 hours of the start of treatment, and it returned to over 50% of normal within the same period after withdrawal of the drug. No gross cumulative effect of the drug has been observed, although in 12% of cases the prothrombin level remained below 50% of normal for periods up to four days after discontinuing the substance.

Apart from slight nausea and vomiting in a small number of subjects, due possibly to the bitter taste of the tablets, no toxic effects were noted.

Transient haematuria, through erroneous overdosage, occurred in one patient, petechial haemorrhage developed in the thrombosed limb in another, and one patient with congestive heart failure developed a haematemesis during treatment.

While under treatment two patients died from causes not attributable to the anticoagulant therapy.

We wish to express our thanks to Professor Sir James Learmonth, Professor R. J. Kellar, Professor W. C. W. Nixon, and Drs. W. D. D. Small, and W. A. Alexander; also to the honorary and unit staff of the Obstetric Unit, University College Hospital, for facilities in carrying out these trials. One of us (C. C. B.) was assistant in the Medical Research Council Clinical Endocrinology Research Unit at Edinburgh during the period of this investigation.

REFERENCES

Allen, E. V. (1947). *J. Amer. med. Ass.*, **134**, 323.
Barnes, A. C., and Ervin, H. K. (1946). *Surg. Gynec. Obstet.*, **83**, 528.
Fisher, R. A. (1948). *Statistical Methods for Research Workers*. p. 121. Edinburgh.
von Kaulla, K. N., and Pulver, R. (1948). *Schweiz. med. Wschr.*, **78**, 806.
Pulver, R., and von Kaulla, K. N. (1948). Ibid., **78**, 956.
Reinis, Z., and Kubik, M. (1948). Ibid., **78**, 785.
Rosicky, J. (1944). *Cas. Lék. ces.*, **83**, 1200.

ISOLATION OF SALMONELLAE FROM DOGS, CATS, AND PIGEONS

BY

J. C. CRUICKSHANK, M.B., Ch.B., D.T.M.
Dip. Bact.

AND

H. WILLIAMS SMITH, Ph.D., M.Sc., M.R.C.V.S.
Dip. Bact.

Member of the Scientific Staff of the Animal Health Trust

(From the Department of Bacteriology, London School of Hygiene and Tropical Medicine)

During the last ten years there has been a great increase in the number of reported outbreaks of food-poisoning in this country, the majority of the cases of the "infection" as opposed to the "toxin" type being due to organisms of the salmonella group. Part of the increase is no doubt due to better diagnosis and reporting, but a considerable proportion is real. Interest in the possible reservoirs and vehicles of salmonella infection has been stimulated, and, in addition to the well-known sources, of which rodents, pigs, cattle, and duck eggs are the most important, certain new sources have been recognized, particularly imported spray-dried egg. The origin of many cases, however, remains obscure, and it seemed of interest to determine whether salmonella infection was prevalent in dogs and cats in view of their close domestic contact with man and his food. Further stimuli to the investigation were the extent to which streets and pavements are fouled with dog faeces, and the frequent observation of cats with free access to unprotected foods in shops. The work was extended to include examination of a number of specimens from pigeons.

Dogs

There are numerous reports of the isolation of salmonellae from dogs suffering from septicaemia, diarrhoea, and other conditions. The most extensive data are provided by Bruner and Moran (1949), who give an account of 2,788 cultures from 32 animal species other than man or fowls studied during a period of 16 years at the Kentucky Experiment Station. The majority of the cultures were from swine, but dogs, with 103 strains, came second on the list. No fewer than 26 different salmonella types were isolated from dogs, of which the most frequent were *Salm. typhi-murium* (accounting for 40% of outbreaks), *Salm. cholerae-suis, Salm. oranienburg, Salm. newport,* and *Salm. anatum.* Bruner and Moran state that salmonella infection is usually a septicaemic condition in lower animals but that asymptomatic carriers may occur. Their work was, of course, not a survey but a record of the identification of organisms submitted from a wide area, probably mostly from sick animals.

Wolff, Henderson, and McCallum (1948) reported the results of the examination of rectal swabs from 100 dogs in a veterinary clinic, an animal shelter, and a crowded insanitary private kennel in Michigan. A remarkably high rate of infection was recorded. Sixteen different salmonella types were isolated from 18 of the dogs; one dog yielded five different types in the course of six swabbings. Many of the dogs had enteritis, or a history of enteritis, but a considerable number were clinically normal at the time of examination. Although the source of the infections was not determined, it is significant that the diet of the animals