For uncomplicated hypertension of Grade 3 or 4 severity, the combination of rauwolfia with a ganglion-blocking agent is the treatment of choice. This may be supplemented by salt restriction, and, if the response is incomplete or delayed, lumbo-dorsal sympathectomy may then be considered. It is said that a previous sympathectomy enhances the effects of hypotensive drugs.

VII. Conclusions

Hypertensive vascular disease presents in such a great variety of patterns, and runs such an unpredictable course, that the effects of treatment are difficult to assess.

Nevertheless, the remedies at present available, if selected judiciously, applied conscientiously, and adjusted carefully to the particular needs of the individual, offer more for hypertensive patients than ever before.

The maintenance of the blood pressure within the normal range permits a reversion of the vascular damage in the retinae, heart, and brain, provided renal function is not grossly impaired and massive tissue destruction has not occurred. Early treatment, even in the severe forms of hypertensive disease, should reduce the risk of serious complications and be the means of prolonging life.

In general, appropriate therapy, which is most often a combination of different remedies, should be determined more by the patient's vascular state than by blood-pressure readings. For the most part the retinal picture, taken with the state of the heart and kidneys, determines treatment.

Summary

A great variety of remedies are available for treatment of hypertensive vascular disease. General measures include: (a) simple psychotherapy; (b) advice regarding a physiological way of life; and (c) instructions regarding weight reduction and restricted Na intake.

The detection of a precise aetiological cause, and its prompt surgical removal, can lead to permanent cure.

In most cases treatment must be determined by the degree of vascular impairment rather than by blood-pressure readings.

All patients showing hypertensive retinopathy should have intensive therapy, also those without serious retinal changes but who have had transient cerebrovascular episodes, attacks of left ventricular failure, or angina. Most patients disabled by severe hypertensive symptoms will also require the more potent hypotensive remedies.

Patients who have no symptoms, or only minor ones, and who present no evidence of serious retinal, cerebral, or cardiac damage require advice about their mode of life, and only mild hypotensive agents such as rauwolfia.

Rauwolfia potentiates other hypotensive agents. Rauwolfia, with or without "veriloid," and a low-salt diet may keep hypertensive heart failure in check, but more commonly pentolinium and Na restriction will be required. Rauwolfia alone or with veriloid often helps the anginal patient with hypertensive coronary disease.

Low-sodium diet combined with veriloid is probably safer than intensive methonium therapy for cerebrovascular lesions. For uncomplicated Grade 3 or 4 hypertension rauwolfia with pentolinium is the treatment of choice.

Preliminary experience suggests that the effective dose of mecamlamine is more readily determined and more constant than that of the methonium compounds. It may therefore ultimately replace pentolinium, but the long-term results are not yet known.

In the presence of uraemia all hypotensive drugs are valueless, though in the milder grades of renal impairment hydrallazine ("apresoline") may prove helpful.

For patients incapable of regulating their pentolinium routine, or if the response to the drug is poor, lumbo-dorsal sympathectomy is indicated, provided renal and cardiac functions are reasonably good.

The accompanying Table and Diet Instructions are reproduced from a *Textbook of Medical Treatment* by permission of the publishers, Messrs. E. and S. Livingstone, Edinburgh.

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DURATION OF ANTICOAGULANT EFFECT IN RELATION TO URINARY EXCRETION OF DEXTRAN SULPHATE

BY

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It has already been reported that when the effect of single equipotent intravenous doses of heparin and dextran sulphate are compared the latter compound shows a slightly greater duration of effect. Comparison of repeated equipotent doses makes this disparity in duration of effect more pronounced (Ricketts *et al.*, 1953). This observation was confirmed by Donzelot *et al.* (1955), who reported that after three to five days' treatment with dextran sulphate the increase in duration of effect of successive doses was such that adequate control of the coagulation time and satisfactory clinical responses in cases of thrombo-embolic disease could be obtained by intravenous dosage at daily intervals or even on alternate days.

One factor which it was thought might be responsible for a cumulative effect of this kind, as compared with the absence of a similar effect with heparin, was a difference between the patterns of urinary excretion of the two drugs. Estimates of the urinary excretion of dextran sulphate in patients treated with dextran sulphate for periods varying from 10 to 23 days, and similar estimates in experimental animals given repeated injections for 10-day periods, were carried out and were related to measurements of the clotting-time of blood and the one-stage "prothrombin" time, in order to investigate this hypothesis. Certain further observations on the clinical effects of dextran sulphate were made incidentally in the course of this investigation.

Materials and Methods

Dextran sulphate ("dexulate," Batch No. 52DS041) was available as a 9% sterile solution, including 0.2% sodium bicarbonate and 0.7% sodium chloride. The dextran sulphate

contained 18.1% sulphur and the solution assayed at 1,200 units per ml. The unit of dextran sulphate is defined as the activity of 0.04 mg. of the standard preparation of dextran sulphate held by the Expert Committee on Biological Standardization of the World Health Organization. The *in vitro* effect of this unit is of approximately the same order of magnitude as the International Heparin Unit.

Estimation of Dextran Sulphate in Urine.—The method of MacIntosh (1941), which depends on the formation of an insoluble purple salt of the dye, toluidine blue, with sulphated polysaccharides, was modified as previously described (Ricketts *et al.*, 1954). Calibration against a dextran sulphate of known sulphur content enabled the results to be expressed as milli-equivalents of ester sulphate per litre of urine (1 mEq of ester sulphate is equivalent to 32 mg. of sulphur).

The blood coagulation time of patients was measured by the method of Lee and White (1913). With rabbits the method of Dale and Laidlaw (1911–12) was employed, using standardized capillary tubes containing steel shot of uniform size. All estimations were performed at 37° C. The "prothrombin" time was estimated by Quick's one-stage method (Quick, 1937) and the results were expressed as a percentage of normal by comparison with a normal serum dilution curve.

Method of Recovery of Dextran Sulphate from Urine.—4.26 g. of cetyltrimethylammonium bromide in 50 ml. of water was added to 2.14 l. of urine estimated to contain 2.13 g. of dextran sulphate. After standing to complete precipitation the insoluble dextran sulphate salt was separated by filtration and centrifugation, and dissolved in an alcohol-chloroform mixture (1:1 by volume). By adding approximately 2 M sodium chloride drop by drop to the clear solution in the organic solvent, sodium dextran sulphate was precipitated as a syrup. This salt was purified by treatment with charcoal, and repeated reprecipitation from alcohol-water solution finally yielded 275 mg. of dextran sulphate containing 18.95% sulphur. The anticoagulant activity of the material recovered was compared with that of the material originally administered to the patient, using the method of Adams (1950) and an assay method previously described (Ricketts and Walton, 1953).

Design of Experiments

Clinical.—Observations were made on five patients who had been admitted to hospital for anticoagulant therapy but who were otherwise unselected. Two were cases of recent myocardial infarction and the other three were cases of venous thrombosis associated with pulmonary embolism. Therapy was initiated by twice- or thrice-daily intravenous injections of dextran sulphate after initial (baseline) values for the coagulation time and prothrombin time had been obtained. The coagulation time was measured before injection ("minimal" coagulation time) and, where necessary, one hour after injection ("maximal" coagulation time). Previous experience had shown that the coagulation-time response to this schedule of intermittent intravenous dosage took the form of a "picket-fence" curve. With thrice-daily injections the peaks (maximal clotting-time) and troughs (minimal clotting-time) of this curve became progressively elevated (Ricketts *et al.*, 1953). In the present investigation, once it was established in a given patient that a cumulative effect of this kind was occurring, the size of the dose and/or the number of injections was reduced in order to produce comparable responses on consecutive days. The general aim was to maintain a minimal coagulation time of at least twice the baseline value to ensure a continuous anticoagulant effect. Observations on the pro-

thrombin-time response were also made but were not used as a guide for the control of dosage. Twenty-four-hour specimens of urine were collected to allow estimation of the excretion of the drug.

Animal Experiments.—The duration of response to single intravenous injections of 750 units per kg. body weight was investigated in two rabbits. A week later the same animals were started on a course of thrice-daily intravenous injections of dextran sulphate, employing a dosage scheme similar to that initially used in the human subjects. The coagulation time was measured each morning before the first injection and again one hour later. Throughout the 10-day period of the experiment the animals were kept in metabolism cages and their urine was collected for each 24-hour period. The animals were maintained on a standard diet and allowed water *ad lib*.

Results

The clinical course of the thrombo-embolic disease in each of the five cases was satisfactory, and in no case was there any evidence of further embolism or thrombosis during therapy.

Coagulation-time Response.—The nature of the response to intermittent intravenous dosage was investigated by the use of different schedules of dosage. Progressive increase of the coagulation time on successive days in response to unaltered dosage, or a similar or increased coagulation time on successive days in response to a diminishing dosage, was taken as evidence of cumulation of dextran sulphate. The minimal coagulation time was regarded as the more sensitive index for this purpose and has been plotted together with the prothrombin percentage and total daily dose in Figs. 1–5. Judged by the nature of the minimal coagulation-time response, an effect ascribable to cumulation of the anticoagulant was observed in Cases 1, 3, 4, and 5. Case 2 presented special circumstances which are discussed separately.

Case 1.—A man aged 51 suffering from myocardial infarction was treated with dextran sulphate for 23 days. It can be seen from Fig. 1 that a daily dosage increasing from 8,400 units on the first day to 25,200 units on the third day raised the minimal coagulation time to the desired level. Evidence of cumulation was then obtained in that, in spite of reduction of dosage to 19,200 units on the fourth and fifth days, the minimal coagulation time continued to rise. Stabilization at the elective level of minimal coagulation

FIG. 1.—Case 1. Response of minimal blood coagulation time and of prothrombin percentage to variation of daily dosage of dextran sulphate.

time (15–20 minutes) was sought by variation of the dose between 13,200 and 3,600 units daily (given as two injections instead of three) during the 6th to the 12th day and by a

FIG. 2.—Case 3. Response of minimal blood coagulation time and of prothrombin percentage to variation of daily dosage of dextran sulphate.

FIG. 3.—Case 4. The minimal blood coagulation time response shows a marked cumulative effect.

three-day period (13th to 15th days) on a steady dosage of 8,400 units. Even this latter dosage resulted in a progressive increase of the minimal coagulation time, and final stabilization was secured on a daily dosage of 7,200 units. During the 23 days of therapy this patient received a total of 235,200 units (17.64 g.) of dextran sulphate.

Case 3.—A woman aged 54 showed clinical and radiological evidence of pulmonary embolism evidently originating from deep vein thrombosis of the right leg associated with a severe superficial phlebitis in the varices of the same leg. An initial dosage of 42,000 units over the first two days raised the minimal coagulation time to the desired level. Reduction of dosage to 13,200 units daily on the fourth to the seventh day produced little variation in the minimal coagulation time until the ninth day, when it showed a rise in level in spite of further reduction of the dose to 12,000 units daily (Fig. 2), suggesting a slower cumulative effect than that seen in the previous case. Dextran sulphate was discontinued on the 10th day, and two days later the patient was transferred to treatment with an oral anticoagulant. The subsequent course of this case is discussed in further detail below.

Case 4.—A man of 50 suffering from a recent myocardial infarction was also found to have chronic bronchitis with purulent sputum. On a daily dosage of 12,000–18,000 units a marked cumulative effect was observed (Fig. 3). The increase in slope of the minimal coagulation time was first noted on the third day and became most pronounced by the ninth day. In this instance, after reduction of the dose on the ninth day, with corresponding reduction in the minimal coagulation time, stabilization was achieved at 15,600 units daily.

Case 5.—A man aged 40 with left femoral vein thrombosis and clinical and radiological evidence of pulmonary embolization was treated with dextran sulphate for 14 days. An

FIG. 4.—Case 5. In this case unvarying daily dosage produced a slow cumulative effect on the minimal blood coagulation time. The reduction in the swelling of the leg affected by venous thrombosis and the fall in the serum cholesterol during therapy are also shown.

unvarying dosage of 18,000 units daily was given for the first three days. Reduction of dosage to 12,000 units on the fourth day (when previous experience had suggested the first evidence of cumulative effect might be anticipated) was not accompanied by a fall in the minimal coagulation time, and more definite evidence of cumulation was obtained when the daily dosage of 18,000 units was resumed (Fig. 4) for the remaining 10 days of this course of therapy.

Effects on Prothrombin Percentage

In all cases the prothrombin percentage was reduced rapidly after injection, and within one hour of the first injection might be as low as 40% of normal. In contrast with the coagulation time the prothrombin percentage did not revert to the baseline level before subsequent injections. The nearest approach was in Case 4, the prothrombin percentage after an initial fall to 45% rising again to 74% 15 hours after the first injection. In every case the prothrombin percentage for most of the therapeutic period lay between 30% and 50%. Fluctuations were less conspicuous than those of the coagulation time, but on the whole the changes in prothrombin percentage and coagulation time mirrored one another. Although, as stated earlier, the prothrombin percentage was not used in the present investigation as a guide to therapy, these observations confirm the contention of Hirschboeck *et al.* (1951) that the prothrombin time can serve as an index of the anticoagulant effect of heparinoids, and it is possible that where both an oral and this intravenous anticoagulant are to be used in sequence it may serve for the control of each drug and for the transition from one to the other. In this latter connexion it was observed that following cessation of therapy with dextran sulphate in Cases 1 and 3 (treated for 23 and 10 days respectively) the coagulation time and the prothrombin percentage did not fall to the baseline values until 48 hours after the last injection.

Urinary Excretion of Dextran Sulphate

Cases 1, 2, and 4 were studied. Some day-to-day variation occurred in the amount of dextran sulphate excreted in the urine in each case, but over the whole therapeutic period the percentage of the total dose accounted for in the urine was close to 40% in all three patients (Table I). No close relation could be seen between the total daily dose and the percentage daily excretion, nor was the latter related to mean daily urinary volume. After the cessation of injections, dextran sulphate continued to be excreted for two to three days, as shown in Table II.

TABLE I

Case No.	Duration of Therapy (Days)	Percentage of Dose Excreted	No. of Days with 30-60% Excretion
1	23	42.6	15
2	16	42.2	12
4	14	39.3	9

TABLE II.—Decline of Excretion at End of Therapy (Figures are Milli-equivalents of Ester Sulphate)

Case No.	Days	Dose	Excreted
4	13	7.28	2.55
	14	7.20	3.82
	15	0	3.73
	16	0	0.66
	17	0	0.23
2	13	5.60	3.50
	14	5.03	1.70
	15	5.60	3.24
	16	1.68	1.11
	17	0	0.06
1	21	3.84	2.10
	22	3.84	0.95
	23	1.92	0.16
	24	0	0.11
	25	0	0.09

Observations in Rabbits

Anticoagulant Effect.—The two animals placed on a dosage regime similar in frequency and spacing of injections to that used in patients showed no evidence of any cumulative effect of the drug. The peaks and troughs of the maximal and minimal coagulation times were comparable in amplitude for a given dose day after day throughout the

course. Even when the size of the dose was increased from the initial 3,600 units daily to 4,800 units daily from the sixth day onwards in rabbit No. 2 (Fig. 5) no evidence of progressive increase in response was found. It should be noted that this absence of cumulation was observed although the animals were given an average dose in excess of 2,000 units per kg. of body weight per day as compared with the cumulative effect observed in man with doses of the order of 200 units per kg. body weight per day.

Fig. 5.—Maximal and minimal coagulation-time responses to dextran sulphate in the rabbit. Note absence of cumulative effect.

Urinary Excretion.—Analysis of the urine showed that the rabbits excreted 66.2% and 58.1% of the total dose administered. Small amounts of dextran sulphate were detected in the urine two to three days after the cessation of injections (Table III), although no anticoagulant effect was observed seven hours after the last injection.

TABLE III.—Decline of Renal Excretion of Dextran Sulphate in Rabbits After Cessation of Injections (Figures are Milli-equivalents of Ester Sulphate)

Rabbit 1			Rabbit 2		
Days	Dose	Excreted	Days	Dose	Excreted
8	2.44	1.290	8	3.05	2.480
9	0.61	0.650	9	0.71	—
10	0	0.084	10	0	2.200
11	0	0.032	11	0	0.046
12	0	0.012			
13	0	0.002			

Examination of Material Excreted

It has been claimed that when heparin is injected the material recovered from the urine, "uroheparin" (Best and Jaques, 1948), differs in physico-chemical characteristics and in biological activity (Marbet and Winterstein, 1951) from the material administered. In a previous study carried out with dextran (³⁵S) sulphate (Ricketts *et al.*, 1954) it was found that during the first hour after intravenous injection of dextran sulphate in the rabbit the material appearing in the urine showed a slightly different R_f value by paper chromatography from that of the material administered. This was assumed tentatively to be explained by the excretion of a low molecular-weight fraction of the injected material.

The present investigation allowed recovery of a larger amount (275 mg., representing 12.9%) of the material appearing in the urine and further examination of its characteristics. On this occasion the material recovered from the urine of Case 1 showed no chromatographic difference from the original material. Its anticoagulant potency *in vitro* and *in vivo* appeared identical with that of the injected drug. This may possibly be accounted for by the fact that the product used in the present work was of more uniform and slightly lower average molecular weight than that used previously.

Other Clinical Effects of Dextran Sulphate

In the three cases with thrombosis of leg veins (Cases 2, 3, and 5), all presenting with painful oedematous limbs, dextran sulphate produced a lessening of pain and a

reduction of the swelling of the affected leg within 48 hours. In Case 5 the circumference of the initially swollen left leg, measured 4 in. (10 cm.) above and below the patella, gradually decreased to equal the corresponding measurements of the unaffected right leg during the 14 days of therapy (Fig. 4). During the first six days, accompanying the reduction of the oedema of the leg, the patient lost 8.5 kg. in weight, suggesting a reabsorption and excretion of about 8.5 litres of oedema fluid during this period.

It had previously been observed that dextran sulphate, like heparin, produces "clearing" of the turbidity of the plasma *in vivo* during alimentary lipaemia (Brown, 1952). The available evidence suggests that this phenomenon is due to the activation of a lipase acting principally on β -lipoprotein, the main carrier of lipids and cholesterol in human plasma. Alteration in the electrophoretic mobility of the lipoproteins following the injection of dextran sulphate has been shown to accompany the clearing phenomenon (Jeavons and Ricketts, 1956). In the present investigation the injection of repeated doses of dextran sulphate was observed to lower the level of plasma cholesterol even where the initial level was not greatly elevated, as in Case 5. The plasma cholesterol was lowered throughout the period of therapy but had reverted to its pre-treatment level 14 days after the last injection (see Fig. 4). Similar observations on the effect of dextran sulphate on plasma cholesterol have been made by Cohen and Tudhope (1956).

Complications and Side-effects of Dextran Sulphate Therapy

The response to dextran sulphate in Case 2 merits more detailed discussion.

Case 2

The patient, a woman aged 45, had previously undergone total hysterectomy and oophorectomy for bilateral ovarian carcinoma. At operation deposits were seen in the pouch of Douglas and pelvic tissues, so a post-operative course of deep x-ray therapy to the lower abdomen to full tolerance was planned one month later. Treatment was given by

FIG. 6.—Case 2. Effect of radiotherapy on platelet count prior to and during dextran sulphate therapy. Note increased sensitivity of minimal blood coagulation time to dextran sulphate therapy during second episode of thrombocytopenia.

wide-field irradiation to a field area 40 by 40 cm. at 230 kV; H.V.L., 2.5 mm. Cu; F.S.D., 100 cm. An incident dose of 3,220 r to each of two fields over a period of 38 days gave a total of 3,500 r.t.d. (roentgen tumour dose). Blood counts were carried out at three-day intervals during treatment, and as the course progressed the blood platelets showed a steady decrease from an initial level of 295,000 per c.mm. to 119,000 per c.mm. by the end of the fourth week (Fig. 6).

On the 28th day of treatment the patient complained of a sudden sharp pain in the chest, and on the following day of pain and swelling in the left leg and thigh, suggesting pulmonary infarction associated with femoral vein thrombosis. Radiotherapy was suspended and treatment with dextran sulphate instituted. Twice-daily doses of dextran sulphate in amounts increasing to 19,200 units daily by the eighth day failed to produce a sustained rise in the minimal coagulation time to the desired level, although an anticoagulant effect was secured for the major portion of each day during this period. The pain in the chest disappeared on the third day of treatment and the pain and swelling in the left leg abated on the fourth day. During the first eight days of dextran sulphate therapy the platelet count rose till it had reached 243,000 per c.mm. The dosage was maintained at 18,000 units. Radiotherapy was resumed from the ninth day. An increasing response of the minimal coagulation time now became apparent and this continued in spite of reduction of dosage to 12,000 units daily (Fig. 6) on the 13th day.

A haematoma appeared at the site of venepuncture on the 15th day and the platelet count was found to have fallen once more to 121,000 per c.mm. Dextran sulphate was discontinued on the 16th day. The coagulation time continued to be elevated for three days. There were no further haematomata and no evidence of bleeding elsewhere was found. Radiotherapy was suspended on the 18th day and the platelets subsequently rose again, but the normal level was not regained for three weeks.

The patient's clinical condition progressed satisfactorily and she was alive and well with no evidence of recurrence of tumour and no further history of thrombo-embolic episodes when seen 18 months later.

Comment

The second fall in the platelet count in this patient was more precipitous than the first (Fig. 6). Whether this was due to an increased sensitivity of the marrow to a second dose of radiation before full recovery had occurred from the effects of the first, or whether the simultaneous administration of dextran sulphate with the second dose of radiation contributed to the thrombocytopenia on the second occasion, are questions it is not possible to decide unequivocally. Thrombocytopenia occurring during intensive therapy with a depot-heparin preparation in 13 out of 30 patients has been reported by Plancherel (1952), and platelet agglutination and thrombocytopenia have been recorded in experimental animals given a variety of sulphate polysaccharides (see Astrup, 1953, for references).

However, Soulier (1955) and Astrup, Flyger, and Gormsen (1955) have confirmed the absence of any direct effect upon platelets of dextran sulphate of the kind used in the present study, as originally reported by Walton (1953). Periodic platelet counts in the other four cases in the present series disclosed no significant changes (Table IV). On balance, therefore, it appeared to us more likely that the thrombocytopenia observed in Case 2 was occasioned by the heavy dosage of radiotherapy.

It was perhaps significant that while the platelet count was rising in this case (during the first eight days) the patient appeared to be relatively resistant to the anticoagulant effect of dextran sulphate, but showed a marked increase in sensitivity to the drug when radiotherapy was resumed and the platelet count again fell to low values. This may be analogous to the increased sensitivity of response to heparin in the presence of thrombocytopenia

TABLE IV.—Platelet Counts During Dextran Sulphate Therapy in Four Patients

Case No.	Total Duration of Therapy	Day of Therapy	Platelet Count
1	23 days	Before	212,000
		2	208,000
		10	183,000
		15	234,000
		23	250,000
3	10 "	Before	307,000
		7	366,000
		11	272,000
		16	230,000
4	14 "	Before	356,000
		2	285,000
		6	310,000
		10	275,000
		16	306,000
5	14 "	Before	204,000
		2	140,000
		5	202,000
		12	106,000
		26	178,000

reported by Conley *et al.* (1948), and emphasizes the necessity for caution in the administration of these anticoagulants to patients with low platelet counts.

Case 3, as described above, was treated with dextran sulphate for 10 days, during which time the oedema of the right leg (due to thrombosis of the deep veins and superficial phlebitis of varices) subsided and no haemorrhagic manifestations occurred. It was decided to continue treatment with an oral anticoagulant (phenindione), and this was instituted three days after cessation of the course of dextran sulphate. On the following day a few red cells were seen in the urinary deposit, and on the next day a profuse haematuria with clot retention occurred. At this time the coagulation time was normal. Subsequent investigation revealed a right-sided hydronephrosis, a mild left hydronephrosis, and a grossly trabeculated bladder associated with uterine prolapse. In view of the interval between the cessation of dextran sulphate administration and the occurrence of the haematuria, and also because of the normal coagulation time when this complication occurred, it seemed unlikely that the haemorrhage could have been caused mainly, if at all, by the dextran sulphate. However, another feature of this patient's subsequent history was possibly related to dextran sulphate therapy. Six weeks after her discharge from hospital (eight to nine weeks after the course of dextran sulphate) she developed a transient partial alopecia from which she had recovered fully two months later. The present case is the first within our own experience to show this side-effect, but Donzelot *et al.* (1955) reported its occurrence in 6 out of the 60 cases they treated with dextran sulphate and it was encountered in 3 out of 11 cases treated by Cohen and Tudhope (1956).

Discussion

The present results confirm and extend previous reports concerning the cumulative effect in human subjects of repeated intravenous injections of dextran sulphate. This cumulative effect is in sharp contrast with the effect of heparin similarly administered in doses of equivalent *in vitro* potency. It is generally agreed that the anticoagulant effect of a single intravenous dose of 5,000 units of heparin lasts two to three hours (Estes and Allen, 1950; Duff, Linman, and Birch, 1951; Brown and Douglas, 1952), and that, to secure a satisfactory clinical and anticoagulant effect, repeated intravenous injections of 10,000 units or more must be given at four- or six-hourly intervals throughout a course of therapy—that is, 40,000 to 60,000 units daily.

In contrast, the present investigation has shown that when a total dosage of 30,000–48,000 units of dextran sulphate is given within the first three to four days, the first evidence of cumulation appears. Thereafter continuous control of the coagulation time and prothrombin time and a satisfac-

tory recovery from the effects of thrombo-embolic disease can be secured with a daily dosage of 8,000 to 16,000 units given in single or divided intravenous doses. Intramuscular injections were not used in our patients, but this route has been employed with apparent success by Donzelot *et al.* (1955). It should be noted that the intravenous dosage scheme now recommended is larger than that used by Donzelot *et al.*, who, using somewhat different criteria of the clinical efficacy of dextran sulphate, obtained results which they regarded as satisfactory by reducing intravenous dosage after a few days to as little as 2,500 units daily or on alternate days, suggesting an even more pronounced difference from heparin in duration of effect.

In considering what might determine this disparity in duration of effect between the two anticoagulants, it is convenient to scrutinize separately the relative proportions of an administered dose which are excreted and retained.

With regard to the proportions excreted, obviously disparity might arise if considerably more of one drug than the other were eliminated. It has been shown by many workers (for references see Jaques *et al.*, 1953) that 40–60% of the dose of heparin administered intravenously can be recovered from the urine in the human subject and in experimental animals. This percentage is very similar to that now found for dextran sulphate. Disparity in duration of effect might also result from selective elimination of qualitatively different fractions of the injected material; for instance, a fraction of small molecular weight and low biological activity in the case of one drug but not in the case of the other. Most commercial heparins are known to be greatly heterodisperse in regard to molecular size and activity (for references see Walton, 1955), and, as mentioned above, evidence has been presented that the fraction recovered from the urine, uroheparin, does differ in physical characteristics and activity from the material injected. In the present experiments, employing the dextran sulphate commercially available, no difference could be detected between the excreted fraction and the original material.

With regard to the portion retained in the body, while it may be assumed that the anticoagulant activity of these drugs is mediated only by the fraction actually present in the circulating blood and free to interact with the "clotting proteins" of the plasma, it is also necessary to consider the distribution of these drugs in the total body fluids. It has long been appreciated that small diffusible molecules injected into the blood rapidly gain access to the extravascular tissue-spaces. It is only in recent years that it has been shown that even relatively large, charged molecules may also pass into the extravascular compartment, and that the concentration in the blood in such instances depends on the establishment of an equilibrium between the concentrations in vascular and extravascular fluid. This concept, generally applied to bodily constituents by Schoenheimer (1942), has been supported, for instance, in the case of individual plasma proteins in experimental animals and man by the work of Whipple and Madden (1944), Miller *et al.* (1949), Sterling (1951), Myant (1952), Gitlin and Jane-way (1953), and others.

In applying this concept to the distribution in the body of polysaccharide sulphates it has seemed reasonable to question whether material of such relatively large molecular size and high electro-negative charge could, in fact, permeate through capillary membranes. But it is known that both heparin and dextran sulphate, when added to plasma, form loose complexes with the plasma globulins as a result of which the charge on the polysaccharide sulphate molecule is partially neutralized. With regard to molecular size, a study of the composition of lymph in animals has shown that in certain regions the blood capillaries are freely permeable to the largest protein molecules and even to small particles (Field *et al.*, 1937). Recently direct evidence of the passage of heparin into the extravascular compartment has been provided by von Kaulla and Pratt (1955), who demonstrated that the concentration in lymph alters in parallel with that in the plasma after intravenous injection,

thus supporting the contention that an equilibrium between heparin levels in vascular and extravascular fluids is accomplished.

The pattern of cumulative effect resulting from repeated injections of dextran sulphate is such as to suggest that if a similar equilibrium between vascular and extravascular compartments occurs, the latter may serve as the main reservoir. This might explain why, early in therapy, when this reservoir is low, the coagulation time reverts rapidly to normal. On the other hand, after a few days' treatment, when the reservoir has been raised, the next injection is followed by a slower fall in plasma concentration and coagulation time. Finally, after the administration of the last dose of a prolonged course, the reinforcement of plasma concentration from the extravascular compartment might explain the slow decline to a normal coagulation time occupying a period of about 48 hours, as compared with an effect lasting only five to seven hours from the same dose at the beginning of treatment.

The rate of inactivation or destruction of the portion of the drug retained in the body must also influence its duration of effect. In the case of heparin it has been suggested that inactivation is effected by an enzyme "heparinase" (Jaques, 1940). However, this enzyme has been isolated only from rabbit liver. An attempt to obtain similar material from beef liver was unsuccessful (Jaques and Keeri-Szanto, 1952). The enzyme from rabbit liver was found to show lack of specificity in that, in addition to acting upon heparin derived from various species, certain synthetic polysaccharides were also degraded. Recently it has been found that a dextran sulphate similar to that used in the present experiments is also susceptible to attack by rabbit heparinase preparations (Jaques and Cho, personal communication).

If, as postulated, the cumulative effect of dextran sulphate in man is accompanied by accumulation in the extravascular compartment, it seems likely that the rate of inactivation or destruction of dextran sulphate must occur more slowly than that of heparin, which, similarly distributed, shows no cumulative effect. Preliminary experiments (Wiegel, Ricketts, and Walton, unpublished) with ^{14}C -labelled dextran sulphate suggest that, in rats, metabolic breakdown (as measured by the appearance of $^{14}\text{CO}_2$ in the expired air) does occur at a slow rate, but direct comparison with the rate of metabolic turnover of heparin in experimental animals or man has not yet been attempted.

Nevertheless, if the inactivation of these anticoagulants in the body is in fact accomplished enzymatically, as the present evidence indicates, it is tempting to suggest that differences between given species in the relative turnover rates of these drugs might be accounted for by differences in the substrate-specificity of their enzymes. Thus the observed disparity in duration of effect of heparin and dextran sulphate in man might be due to a more marked specificity of the human enzyme for the "natural" substrate, heparin, which is accordingly degraded more quickly and completely than the synthetic analogue dextran sulphate. On the other hand, the similar duration of effect of the two drugs in the rabbit might be due to the ability of rabbit heparinase to degrade both anticoagulants with equal facility.

Whatever the mechanism involved, it is clear that the cumulative action of dextran sulphate in man confers upon this drug certain immediate practical advantages over heparin for anticoagulant therapy. These advantages include lessened cost, reduced discomfort to the patient, and reduction in the number of man-hours of skilled medical attention necessary for the actual administration of the anticoagulant, all of which accrue from the longer spacing of injections which is possible once the patient is stabilized at the selected level of response. These advantages become more marked where treatment is given for prolonged periods.

On the other hand, prolonged therapy with dextran sulphate, as with heparin, appears to be accompanied by the occurrence of transient alopecia as a late sequel in some patients. A survey of 105 cases known to us to have been

treated with dextran sulphate suggests that this side-effect occurs in about 10% of cases, affecting males and females alike. However, in cases treated early in the series, since we were unaware of the time-lag that may occur before the alopecia develops, it is possible that cases were overlooked because the follow-up was not long enough. Estimates of the incidence of alopecia following treatment with other polysaccharide sulphates vary from 19% ("treburon"—Hirschboeck *et al.*, 1954) and 70% ("thrombocid"—May, 1951) to 54–66% for heparin (Merz, 1950) and 78% for heparin or thrombocid given in conjunction with dicoumarol (Fischer *et al.*, 1953).

In addition to its use as an anticoagulant for established cases of thrombosis, heparin has been advocated recently for prophylaxis and treatment in conditions associated with atheromatosis and accompanied by abnormally high plasma cholesterol and lipoprotein levels (Graham *et al.*, 1951). The biochemical abnormalities are said to be corrected and the clinical condition is improved thereby.

Preliminary observations suggest that dextran sulphate is similarly effective in "clearing" lipaemic plasma, altering the distribution of molecular species of lipoproteins and their electrophoretic mobilities, and lowering the plasma cholesterol, but that these effects occur with greater intensity and for a longer duration than the changes occurring with similar doses of heparin. Further work on this aspect of the clinical use of dextran sulphate will be reported later.

Although, in this series, alopecia was the only side-effect encountered which appeared to be directly related to dextran sulphate therapy, it must be emphasized that the accumulated experience with this anticoagulant is still small compared with that accruing from the extensive use of heparin. It is therefore still necessary to advocate careful clinical and pathological investigation of all cases in which dextran sulphate is used.

Moreover, although the overall anticoagulant effect of dextran sulphate resembles that of heparin, there is evidence that these two anticoagulants show both quantitative and qualitative differences when compared by a variety of methods (Mussett and Perry, 1955; Grasset and Schwartz, 1955; Astrup *et al.*, 1955; Soulier, 1955), due to differences in the nature of their interventions in the blood-coagulation mechanism (Forwell and Ingram, 1956). This suggests that further clinical experience will be necessary before a final evaluation can be made of the place of dextran sulphate in the treatment of thrombo-embolic disease.

Summary

Dextran sulphate, administered by intermittent intravenous injections to five patients suffering from thrombo-embolic disease, was found to have a marked cumulative effect when treatment was prolonged beyond three to five days. As a result reduction in the size of dosage and/or lengthened spacing of injections could be effected without materially affecting the clinical efficacy of dextran sulphate as an anticoagulant.

The cumulative effect in man could not be accounted for on the basis of the urinary excretion of a smaller percentage of the injected dose than that of heparin, the naturally occurring analogue of dextran sulphate.

A dosage schedule of dextran sulphate, similar in spacing of injections but larger in relation to body weight, did not produce a cumulative effect in rabbits. In this species a slightly larger proportion of the injected dose was excreted in the urine than the proportion found in man.

It is suggested that in man dextran sulphate differs from heparin in duration of effect because of a slower rate of metabolic breakdown in the body and consequent accumulation, probably in extravascular fluid.

In addition to its direct effect upon the blood-coagulation mechanism, dextran sulphate relieved oedema in cases of thrombophlebitis and produced effects similar to those of heparin on the plasma lipoproteins and plasma cholesterol.

Our thanks are due to Professor A. P. Thomson for his interest in this study, to Drs. E. Bulmer, C. F. Hawkins, G. M. Holme, and Clifford Parsons for permission to treat and publish details of cases under their care at the General Hospital and Queen Elizabeth Hospital, Birmingham. We are indebted to Dr. M. J. Meynell for determination of the prothrombin time and other pathological investigations and to Dr. H. A. Ellis for clinical assistance. The dexulate used in this investigation was generously supplied by Messrs. Glaxo Ltd. This work was carried out while one of us (S. M. J.) was in receipt of a grant from the Medical Research Committee, United Birmingham Hospitals Endowment Fund.

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"Whatever the cause, it has been remarked at post-natal clinics how women at this stage [the early post-natal months] may indulge in very acrimonious comments about their husbands, especially in relation to their sexual demands. But this hostility, though virulent, is transient. Surely at this stage there is much conflict in the wives' minds, and they and their husbands need both support and comfort from society, rather than a demand that they should evince a bliss which theory and tradition expect. 'She's got everything she wants . . . a husband, a baby, a new home. She must be in her seventh heaven. What more can she want?' This style of comment only adds to the burden of guilt, when discontent arises. It may also induce the girl to wonder if she is abnormal, in that she cannot throw off anxiety and tension, and assume the glow which is the *sine qua non* of young mothers in magazines! Possibly now the first bitterness is sown. Certainly by the time the first child is 3 or so, it is quite common for the parents to need matrimonial guidance."—Mrs. HEIGER writing in the October issue of *Marriage Guidance*.

DEXTRAN SULPHATE: USE AS AN ANTICOAGULANT, AND ACTION IN LOWERING SERUM CHOLESTEROL

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The sulphuric esters of several polysaccharides of high molecular weight possess an anticoagulant action similar to that of heparin (Chargaff *et al.*, 1936; Astrup *et al.*, 1944). Many of these compounds cause precipitation of fibrinogen (Astrup and Piper, 1946) and agglutination of platelets (Piper, 1945), and when injected into animals lead to intractable bleeding (Astrup *et al.*, 1944). Walton (1951, 1952, 1953, 1954) showed that dextran sulphates of high molecular weight had similar biological actions but that compounds of smaller molecular weight (about 7,500) caused no such toxic effects. Ricketts (1952) prepared and studied the properties of a series of sulphuric esters of dextran which differed in molecular weight and sulphur content. Dextran sulphate of molecular weight of about 7,500 was non-toxic, but toxicity developed with increasing molecular weight. Anticoagulant activity was independent of molecular weight but depended on the number of sulphate groups for each glucose unit. Ricketts and Walton (1953) prepared a dextran sulphate which had anticoagulant action but was not toxic.

A clinical trial of dextran sulphate of optimum molecular weight and sulphur content showed that this substance was an effective anticoagulant and had an action similar to but more prolonged than that of heparin (Ricketts *et al.*, 1953).

We report the effect on the clotting-time of single and repeated intravenous injections of dextran sulphate sodium ("dexulate") and describe the use of this drug in the treatment of thrombotic disease. The change in serum total cholesterol during prolonged treatment with dextran sulphate is also described.

Methods

Measurement of Clotting-time.—By venepuncture 2 ml. of blood was withdrawn, using a sterile dry syringe, and approximately 0.5 ml. of blood was added to each of three clean, dry test-tubes, 0.75 cm. internal diameter. The test was timed from the entry of blood into the syringe. The tubes were at once placed in a water-bath at 37° C., and at intervals of one minute each tube was lifted and tilted through 90 degrees. When the blood did not flow on tilting, the tube was inverted and gently shaken, to break the film of clot on the surface of the blood. The end-point was reached when no blood flow occurred with this manoeuvre. The average of the results from the three tubes was taken as the clotting-time. In the control of dextran sulphate therapy this method of estimating clotting-time was more satisfactory than determining the end-point of clotting by tilting to 90 degrees only. By the latter method lower values were obtained, which did not give a reliable guide to the control of dosage. In two patients an attempt was made to control therapy by clotting-times measured without inversion