Summary

The various methods of estimating the E.S.R. employed in the Edinburgh group of hospitals have been investigated and found wanting. A new method has been suggested, employing sequestrene as the anticoagulant and using a suspended Westergren pipette in place of the static pipette usually employed. This new method has been checked and found satisfactory, and has been in use over the past 18 months in the Royal Infirmary, Edinburgh.

It is with great pleasure that I acknowledge the help and advice of so many people—in particular, Sir Stanley Davidson, my recent chief; Dr. J. Richmond; Dr. A. Doig and other senior members of his staff; Mr. Sklaroff, statistician at the Usher Institute; Mr. R. Samson and Miss F. McNulty, laboratory technicians; and the secretaries, Misses Williams, Potts, and Webster, and other members of the unit.

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ORTHO-CRESOTINATE AND DIABETES MELLITUS

BY

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Aspirin in full therapeutic doses relieves mild diabetic symptoms, lowers the fasting blood-sugar level, and reduces glycosuria and ketonuria in patients with mild to moderately severe diabetes mellitus (Reid, Macdougall, and Andrews, 1957). One of the disadvantages of aspirin therapy, however, is the fairly frequent occurrence of undesirable side-effects, such as tinnitus and deafness, with the high dosage of the drug required to achieve satisfactory control of the diabetes.

Ortho-cresotinic acid.

Stockman (1912) observed that sodium ortho-cresotinate (see Formula) was as effective as sodium salicylate in treatment of rheumatic fever, but even with

high dosage it did not cause deafness or tinnitus. The effect of ortho-cresotinate in diabetes mellitus has been investigated to find out whether it has a similar hypoglycaemic action to that of salicylate without its undesirable side-effects.

Nine patients with diabetes mellitus were treated with ortho-cresotinate, and clinical particulars are shown in Tables I and II.

TABLE I.—Clinical Particulars of Group 1. Patients Previously Treated by Dietary Restriction Alone

Case No.	Age	Sex	Weight (kg.)	Duration of Diabetes	In-patient Diet	
					g. Carb./Day	Total Cals./Day
1	68	F	57.7	3 years	150	1,600
2	59	F	72.9	1 month	120	1,300
3	60	F	71.5	8 years	150	1,500
4	57	F	56.5	2 "	150	1,700
5	68	F	52.9	3 months	150	1,700
6	55	M	59.5	3 "	148	1,600

TABLE II.—Clinical Particulars of Group 2. Patients Previously Treated by Diet and Insulin

Case No.	Age	Sex	Weight (kg.)	Duration of Diabetes	Diet		Insulin Requirement (Units/Day at End of Control Period)
					g. Carb./Day	Total Cals./Day	
7	49	F	56.5	1 year	195	2,100	28 I.Z.S.
8	49	F	50.7	10 years	195	2,100	50 "
9	37	M	79.9	6 "	200	2,000	54 "

The first group of six patients (group 1) had been treated previously by dietary restriction only for periods varying from one month to eight years, but control of the diabetes had not been satisfactory, as indicated by the occurrence of mild diabetic symptoms in five of the patients, and the presence of persistent glycosuria and elevated blood-sugar levels in every patient. Two patients (Cases 2 and 3) were overweight at the beginning of the investigation; the other four were at or below their optimum weight. The second group of three patients (group 2) had already been treated by diet and insulin before the investigation. Their insulin requirement when admitted to hospital ranged from 28 to 88 units of insulin zinc suspension (I.Z.S.) daily.

Methods of Investigation

All patients were admitted and detained in hospital for the duration of the investigation. Treatment was controlled and the effect of the drug determined by frequent estimations of the fasting blood-sugar and serum ortho-cresotinate levels and daily measurement of sugar and ketone output in 24-hour urine. Every patient in group 1 was first treated by dietary restriction until the fasting blood-sugar was stable. This control period varied from 7 to 13 days. The daily carbohydrate intake, ranging from 120 to 150 g. was kept constant throughout both the control and treatment periods. The diet contained 65 to 79 g. of protein, and 65 to 85 g. of fat, and the total calorie content ranged from 1,300 to 1,700 a day. At the end of the control period of dietary restriction the patients were given a course of ortho-cresotinate which lasted for at least two to three weeks.

The three patients in group 2 were treated initially by dietary restriction and insulin for a control period of 10 to 24 days. During this time the daily dosage of insulin was altered if necessary to stabilize the fasting blood-sugar. After the control period a course of ortho-cresotinate, lasting for two to three weeks, was started and insulin administrations continued. The dose of

insulin was then gradually reduced, if indicated, by a reduction in fasting blood-sugar and glycosuria. In this way the amount of insulin replaced by the course of the drug was determined for each patient.

The diets which the three patients requiring insulin had taken as out-patients were continued in two, but in the remaining patient the daily carbohydrate intake was reduced from 287 to 200 g. Throughout the investigation the diets were constant in carbohydrate content, which ranged from 195 to 200 g. daily, with total calorie content of 2,000 to 2,100 calories a day: the protein and fat contents ranged from 85 to 93 g. for protein and 92 to 123 g. for fat each day.

The drug was given as a solution of sodium ortho-cresotinate to one patient and as acetyl-ortho-cresotinate in the form of tablets to the others. In all cases it was given four-hourly during the day, omitting one dose in the middle of the night. Actual dosage, which ranged from 1 to 1.3 g. four-hourly, was controlled by frequent estimations of serum ortho-cresotinate level (Trinder, 1954) to maintain a serum level of 30 to 35 mg./100 ml. The doses of the drug required and the mean serum ortho-cresotinate level for individual patients, which varied from 27 to 40 mg./100 ml., are shown in Table III.

TABLE III.—Dose of Ortho-cresotinate and Serum Ortho-cresotinate Level

Case No.	Weight (kg.)	Maintenance Dose (g. given 5 Times/Day)	Serum Ortho-cresotinate Level (mg./100 ml.)	
			Range	Mean
1	57.7	1.0	25-28	27
2	72.9	1.0	29-34	32
3	71.5	1.0	26-38	34
4	56.5	1.2	28-35	32
*5	52.9	1.2	32-39	35
6	59.5	1.0	27-41	35
7	56.5	1.3	36-46	40
8	50.7	1.2	31-48	40
9	79.9	1.3	35-45	40

* This patient was treated with sodium ortho-cresotinate; all others with acetyl-ortho-cresotinate.

Response to Ortho-cresotinate

The response to treatment of the six patients in group 1 is considered first and results are presented in Tables IV, V, and VI.

Symptoms.—When admitted, five of the six patients treated by dietary restriction alone complained of mild diabetic symptoms, five complained of nocturia, two of thirst, one of pruritus vulvae, and one of polyuria; the other patient was symptom-free. By the end of the control period of dietary restriction three patients had become symptom-free, and by the end of the course of ortho-cresotinate all patients were symptom-free, with the exception of one who still complained of thirst and polyuria. This patient continued to produce large volumes of urine of low specific gravity, though the fasting blood-sugar level was normal and there was no glycosuria.

Fasting Blood Sugar.—Fasting blood-sugar levels were estimated three to four times a week by Lehmann and Silk's (1952) modification of Folin and Wu's method. At the end of the dietary control period all six patients still showed stable and elevated fasting blood-sugar level. A reduction in fasting blood-sugar level was noted in every patient during the course of ortho-cresotinate—the level falling to within normal limits in five patients and to 145 mg./100 ml. in the sixth patient by the 14th and 15th day of treatment.

The mean value for the fasting blood sugar in the six patients fell from 208 mg./100 ml. on the day of starting the course to 114 mg./100 ml. by the 14th to 15th day of treatment. The significance of the difference between the means was determined by Student's *t*-test. The fall in blood sugar by the 14th to 15th day of treatment was found to be highly significant ($P < 0.001$). The fasting blood-sugar levels during the last three to four days of control period and during the period of treatment are shown in Table IV.

TABLE IV.—Effect of Ortho-cresotinate on Fasting Blood-sugar Level of Patients Previously Treated by Dietary Restriction Alone

Case No.	Fasting Blood Sugar (mg./100 ml.)				
	Before Ortho-cresotinate		During Ortho-cresotinate		
	Day 3-4	Day 0-1	Day 3-4	Day 7-8	Day 14-15
1	200	193	152	126	117
2	193	215	228	138	109
3	204	234	148	163	145
4	183	165	110	121	95
5	237	233	145	147	115
6	186	208	128	111	105
Mean ..	201	208	152	134	114

Glycosuria.—The total urinary sugar output in 24 hours was estimated daily throughout the period of investigation by Benedict's (1911) method and the results were expressed in g. of glucose per 24 hours. Glycosuria was present in five of the six patients by the end of the control period. The only patient not showing glycosuria by that time was one who had already had a short course of cresotinate of seven days' duration, which had to be discontinued because of side-effects, the drug being restarted five days later. During the period of treatment with ortho-cresotinate there was a reduction in glycosuria in five cases and the urine became sugar-free in four patients. The other two patients still showed a small amount of reducing substance in the urine. The mean value for 24-hour urine-sugar output fell from 19.8 g./24 hours at the start of treatment to 2.5 g./24 hours on the 14th to 15th day of treatment. This small quantity of reducing substance may be accounted for by excretory products of ortho-cresotinate in the urine which reduce Benedict's solution. For the five patients who still showed glycosuria at the end of the control period the significance of the difference between the means was determined by Student's *t*-test. The fall in glycosuria by the 14th to 15th day of treatment was found to be just significant ($P > 0.02$ but < 0.05). The results for individual patients are shown in Table V.

TABLE V.—Effect of Ortho-cresotinate on Glycosuria

Case No.	Total Urine Sugar (g./24 Hrs.)				
	Before Ortho-cresotinate		During Ortho-cresotinate		
	Day 3-4	Day 0-1	Day 3-4	Day 7-8	Day 14-15
1	16	19	0	0	12.0
2	26	40	14	1	0
3	46	41	10	6	0
4	18	0	0	13	0
5	11	12	10	2	3
6	10	7	0	0	0
Mean ..	21.2	19.8	5.7	3.7	2.5

Ketonuria.—Ketones in the urine were estimated by the method of Greenberg and Lester (1944). The urine ketones were measured quantitatively only if the urine gave a positive result to Rothera's test. Only two patients had ketones present in the urine on admission, and in both cases the ketonuria disappeared during the

control period. Slight ketonuria occurred in one patient during the course of ortho-cresotinate but lasted for only one day.

Oral Glucose-tolerance Tests.—Oral glucose-tolerance tests were carried out on all six patients during the control period just before treatment was started and again on the 13th to 14th day of the course of ortho-cresotinate. 50 g. of glucose was given orally with the patients fasting, and the blood-sugar levels were estimated when fasting and at $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2 hours after administration of the glucose. The results of these tests are shown in Table VI. The blood-sugar curves on the 13th to 14th day of the course of ortho-cresotinate were all similar but at a lower level than those obtained before treatment was started.

TABLE VI.—Oral Glucose-tolerance Test—50 g. Glucose (Orally When Fasting)

Case No.	Blood Sugar (mg./100 ml.) After Glucose									
	Fasting		$\frac{1}{2}$ Hour		1 Hour		$1\frac{1}{2}$ Hours		2 Hours	
	Pre-Cres.	Day 13-14	Pre-Cres.	Day 13-14	Pre-Cres.	Day 13-14	Pre-Cres.	Day 13-14	Pre-Cres.	Day 13-14
1	193	117	222	228	281	228	333	242	333	255
2	215	109	254	175	330	196	339	214	339	182
3	234	145	333	220	337	280	382	280	371	212
4	165	95	270	237	—	237	310	244	310	244
5	233	137	278	237	356	259	356	208	311	185
6	208	105	254	168	346	217	362	246	330	224
Mean	208	118	285	211	330	236	347	239	332	217

The response to the drug of the three patients in group 2 who required insulin is now considered.

Case 7

A woman aged 49, with diabetes of one year's duration, remained well stabilized and controlled while receiving 195 g. of carbohydrate and a dose of 28 units of I.Z.S. daily. During ortho-cresotinate therapy the dose of insulin was reduced over a period of five days and then discontinued altogether, the diabetes remaining just as well controlled by ortho-cresotinate alone as by insulin therapy when judged by fasting blood-sugar levels. The fasting blood-sugar remained within normal limits throughout the period that ortho-cresotinate treatment replaced insulin therapy. Glycosuria, which was slight and intermittent during the control period with insulin, showed a slight increase during cresotinate therapy. There was no ketonuria during the control or treatment periods. The oral glucose-tolerance test when the patient was receiving insulin alone and then on ortho-cresotinate alone showed similar curves, but the level was slightly lower during ortho-cresotinate therapy. In this patient 28 units of I.Z.S. was replaced by ortho-cresotinate therapy, and the diabetes was satisfactorily controlled.

Case 8

A woman aged 49, who had had diabetes for 10 years, had also suffered from rheumatoid arthritis for the past three years, and this was active when she was admitted. Her diabetes had not been well controlled as an out-patient, as shown by symptoms of increased frequency of micturition, nocturia, and considerable glycosuria. Her insulin requirement as an out-patient had been 16 units of I.Z.S. (amorphous) and 72 units of I.Z.S. daily, but by the end of the control period the insulin requirement had fallen to 50 units of I.Z.S. daily, presumably owing to stricter dietary control while in hospital. Marked fluctuation in fasting blood-sugar level with considerable variation in degree of glycosuria continued through the control period and indicated an unstable state of diabetes mellitus.

During ortho-cresotinate therapy the dose of insulin was reduced to 12 units of I.Z.S. (amorphous) plus 32 units of I.Z.S. daily, and after cresotinate had been discontinued the

insulin requirement was in the range of 12 units of I.Z.S. (amorphous) plus 52 units of I.Z.S. to 16 units of I.Z.S. (amorphous) plus 56 units of I.Z.S. daily. The fall in insulin requirement during cresotinate treatment was slight, amounting to 20 units of I.Z.S. daily, but the fluctuation in fasting blood-sugar and glycosuria was just as pronounced during combined insulin and cresotinate treatment as with insulin alone.

Case 9

A man of 37 had suffered from diabetes mellitus for six years. His diabetes had not been well controlled during the six months prior to admission and he had experienced frequent hypoglycaemic attacks, often without any warning. As an out-patient he had taken a diet of 287 g. of carbohydrate daily, with an insulin dosage of 88 to 92 units of I.Z.S. daily. After admission to hospital the diet was reduced to 200 g. of carbohydrate, with a total of 2,000 calories, and this was then strictly adhered to throughout the control and drug-treatment periods.

During the control period further hypoglycaemic attacks were experienced and the dose of insulin was reduced to 54 units of I.Z.S. daily. Marked fluctuations in fasting blood-sugar level and degree of glycosuria occurred and there was intermittent ketonuria throughout the control period.

During ortho-cresotinate therapy the insulin dose was reduced and he was stabilized by 28 to 36 units of I.Z.S. daily. His insulin requirement thus fell by about 18 units of I.Z.S. daily during the course of ortho-cresotinate. Though considerable variations in fasting blood-sugar, glycosuria, and ketonuria continued during the course of cresotinate, no actual hypoglycaemic symptoms were experienced during combined drug-insulin therapy. Combined insulin and ortho-cresotinate therapy was continued as an out-patient, and he has been working regularly for a year. During this time he has experienced only occasional hypoglycaemic symptoms, and he now also gets adequate warning of attacks. The striking point about this patient was not so much the amount of insulin replaced by ortho-cresotinate as the virtual elimination of troublesome hypoglycaemic attacks. Undoubtedly combined insulin and ortho-cresotinate therapy was more satisfactory than treatment with insulin alone.

Incidence of Side-effects

Of the nine patients treated with ortho-cresotinate, seven experienced side-effects from the drug. The most common symptoms were anorexia, nausea, headache, and vomiting. These symptoms occurred mainly during the first week of the course (2nd to 8th day) and then tended to pass off. Two patients had symptoms of headache, dizziness, anorexia, nausea, and vomiting severe enough to cause temporary discontinuance of the drug, and relief of these symptoms occurred within one to two days. When treatment was restarted a few days later a full 14-days course of the drug was given. During this second course only slight temporary headache was experienced by one patient, and the other remained symptom-free. Smaller doses of the drug were given at the beginning of the second course, and this resulted in a slower rise in the serum ortho-cresotinate level. These symptoms were due to overdosage with the drug and were associated with high serum ortho-cresotinate levels. They occurred especially in patients when there was a rapid initial rise in serum ortho-cresotinate level and could be avoided by a more gradual build-up of the serum concentration of the drug. The toxic effects were all temporary except for persistent anorexia in one patient when the serum level was 40 mg./100 ml. Side-effects occurred mainly when the serum ortho-cresotinate levels were 35 mg./100 ml. or over, though in some

patients they occurred at as low a level as 32 mg./100 ml., and other patients were symptom-free at levels of 40 to 45 mg./100 ml.

The outstanding feature about these results was the complete absence of deafness or tinnitus in every patient during treatment with ortho-cresotinate.

Body Weight.—Weight changes during the control period ranged from a gain of 0.7 kg. to a loss of 3.7 kg. During treatment with ortho-cresotinate two patients showed a slight increase in weight and the others lost from 0.4 to 4.6 kg. It was the patient whose diet was reduced from 287 to 200 g. of carbohydrate daily who lost 4.6 kg.

Discussion

These results show that ortho-cresotinate has a similar hypoglycaemic action to aspirin in diabetes mellitus. A disadvantage of aspirin therapy in diabetes mellitus is the fairly frequent occurrence of side-effects with the high dose of the drug required to cause a maximum hypoglycaemic effect. The annoying symptoms of deafness and tinnitus often occur during treatment with aspirin, but neither of these symptoms was experienced by any of the patients treated with ortho-cresotinate.

The drug was given with the aim of attaining a serum cresotinate level of 30 to 40 mg./100 ml. and the serum levels during the course were in the range of 27 to 40 mg./100 ml. for individual patients. With these doses there was a high incidence of temporary side-effects from the drug, the most common being headache (3 patients), anorexia (4 patients), nausea (5 patients), vomiting (3 patients), dizziness (3 patients, and drowsiness (1 patient). These side-effects were due to overdosage, and their occurrence indicates that ortho-cresotinate therapy, like aspirin, requires to be controlled initially by estimations of serum level of the drug until a suitable dose has been determined for each patient. This is necessary because of the variation in the dose required by individual patients to attain the same serum level of the drug. This means that it is advisable to admit patients to hospital to start ortho-cresotinate therapy until the precise dose for each individual has been determined. A slow build-up of dose is recommended to prevent the development of undesirable side-effects.

Summary

The effect of ortho-cresotinate in diabetes mellitus has been investigated in nine patients by administering the drug for two to three weeks after an initial control period. In the six patients previously treated by diet alone a course of ortho-cresotinate relieved mild symptoms of diabetes and caused a fall in fasting blood-sugar and a reduction in glycosuria in all cases. During a course of the drug in the other three patients already being treated with insulin the insulin requirement of all three patients was reduced and in one patient 28 units of insulin zinc suspension daily was replaced by ortho-cresotinate therapy with satisfactory control of the diabetes.

No deafness or tinnitus was experienced by any of the patients, and the most frequent side-effects which occurred in seven patients were headache, anorexia, nausea, and vomiting. These were all temporary, and could be avoided by slowly increasing the dose until the required amounts for each individual patient had been reached.

Ortho-cresotinate has a similar hypoglycaemic effect to aspirin in patients with mild to moderately severe diabetes mellitus without the undesirable side-effects of deafness and tinnitus.

Our thanks are due to Miss M. McCombie, of the dietetic department of the Western Infirmary, for supervision of the diets, and to the staff of the unit for invaluable technical assistance. We are especially indebted to Dr. C. J. W. Brooks for preparing acetyl-ortho-cresotinate.

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TWO TYPES OF LUPUS ERYTHEMATOSUS CELL FACTOR, SHOWN BY INDUCED L.E. CELL PHENOMENON IN DOGS

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In a previous paper (Bencze, 1960) it was shown that typical lupus erythematosus (L.E.) cell formation can be induced in dogs by transfusion of systemic lupus erythematosus (S.L.E.) plasma. Similar investigations completed on hospital patients gave evidence of the existence of transferable and non-transferable L.E. plasma factors (Bencze, Kovács, and Cserhádi, 1959).

The present communication reports on two types of L.E. plasma factor, as shown by the results of transfusing S.L.E. plasma in experiments on dogs. The experiments were done according to the technique described previously (Bencze, 1960).

Results

The plasma containing L.E. cells from nine patients with typical signs of S.L.E. was transfused into 17 dogs (Table I). The introduction of plasma from three S.L.E. patients produced the L.E. cell phenomenon in the dogs. On the other hand, plasma from six S.L.E. subjects failed to induce the L.E. cell phenomenon.

Further human and animal experiments were carried out with plasma containing L.E. cells from 11 donors (Table II). Plasma of six S.L.E. patients was transfused into human subjects as well as into dogs; plasma from five S.L.E. donors was transfused either into human subjects or dogs, but not both. The plasma from six S.L.E. patients gave identical results in man and dog. The transferable L.E. cell factor produced the L.E. cell phenomenon both in man and in dog (Table II, lines 1 and 2). In four cases the L.E. plasma factors proved