RESEARCH PAPERS

THE EFFECT OF PENICILLIN AND OF STREPTOMYCIN ON BLOOD COAGULATION IN NORMAL SUBJECTS

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INTRODUCTION

A NUMBER of workers have presented laboratory evidence that penicillin and streptomycin affect the clotting of blood, and it has been suggested that recumbency thrombosis might be precipitated by their use. A study of the literature shows a conflict of experimental evidence, and it appears that much of the reported work has been imperfectly controlled. It was therefore thought worth while to reinvestigate this problem in healthy human volunteers by using an experimental design in which the effects of person-to-person and day-to-day differences would not bias the results, and to study a number of clotting tests.

REVIEW OF THE LITERATURE

The experimental literature is summarised in Table I. Impressions of corresponding clinical effects have been equally varied. Frada,[1] Courty and Biscaye[2] and Ochsner *et al.*[3] suggested that penicillin treatment might contribute to the occurrence of thrombosis in ill persons, while, on the

TABLE I

SUMMARY OF THE LITERATURE

These reports deal with effects in man and in laboratory mammals: the tabulation does not refer to the *in vitro* experiments which some of the authors also describe

Drug	Effect reported		
	Coagulant	Anticoagulant	None
Penicillin	Moldavsky *et al.*[32] Macht[20,21,22]	Hines and Kessler[4] (potentiation of heparin)	Ungar[33] Lewis[34] (including hæmophilia) Macht and Ostro[23] (hæmophilia) Weiner *et al.*[35] Dolkart *et al.*[36] (including tests with heparin) Reggianini[37]
Streptomycin	Macht (*ibid.*) Donatelli and Pasquinucci[38] Meneghini *et al.*[39]	Giannico and Provini[40]	Farrington *et al.*[41] Nassi and Ulivelli[42] de Michele and Portella[43] Elson[44]

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other hand, Hines and Kessler[4] thought that penicillin might contribute to the occurrence of hæmorrhage in cases of subacute bacterial endocarditis under combined treatment with penicillin and heparin.

EXPERIMENTAL

3 experiments were made, the drugs being given parenterally. The first 2, each with 6 subjects, investigated penicillin and streptomycin separately and were similar in design. The third, with 2 sets of 4 subjects, included both drugs.

1. *Experiments* 1 *and* 2

Drug Administration

Experiment 1: *Penicillin.* Tests were made about 15 minutes after a single injection of 0·5 mega-units of crystalline sodium penicillin, and again 12 to 24 hours after the last of 4 daily injections of 0·6 mega-units of crystalline procaine penicillin G.

Experiment 2: *Streptomycin.* Tests were made about 15 minutes after a single injection of 1·0 g. of dihydrostreptomycin sulphate, and again 12 to 24 hours after the last of 3 daily injections of the same quantity given on the days following the first test.

The first series of tests with each drug was designated "immediate," and the second series "delayed."

TABLE II

PAIRING OF DRUG AND CONTROL SUBJECTS IN EXPERIMENTS 1 AND 2

The same design was used with different subjects in the two experiments

Week and day of test	Test	Drug subject	Control subject
Week 1. Monday Week 1. Friday	Immediate Delayed	I	IV
Week 2. Monday Week 2. Friday	Immediate Delayed	II	V
Week 3. Monday Week 3. Friday	Immediate Delayed	III	VI
Week 4. Monday Week 4. Friday	Immediate Delayed	IV	
Week 5. Monday Week 5. Friday	Immediate Delayed	V	II
Week 6. Monday Week 6. Friday	Immediate Delayed	VI	III

Controls. It was not practicable to test all 6 subjects on each experimental day, so they were tested in pairs according to the scheme of Table II. In both experiments the six subjects thus each provided a "drug" and a "control" reading in the immediate and in the delayed tests, with an interval between the drug and control readings on any one subject. The paired tests were made strictly in parallel.

In these experiments the control subjects did not receive inert injections.

Tests Used

(i) *Clotting tests. The whole blood clotting time* was measured on venous blood by the method of Lee and White[5] at 37° C. in ordinary glass

tubes 6·5 × 0·9 cm. and in similar tubes silicone-coated (in both cases taking the mean clotting time from 4 tubes at each venepuncture), and on capillary blood by the bead-capillary method of Dale and Laidlaw,[6] taking the mean of single readings obtained from a stab wound in each ear lobe.

The "prothrombin consumption" was measured 1 hour after venepuncture on the "glass" and "silicone" sera from the Lee-White tubes, by observing

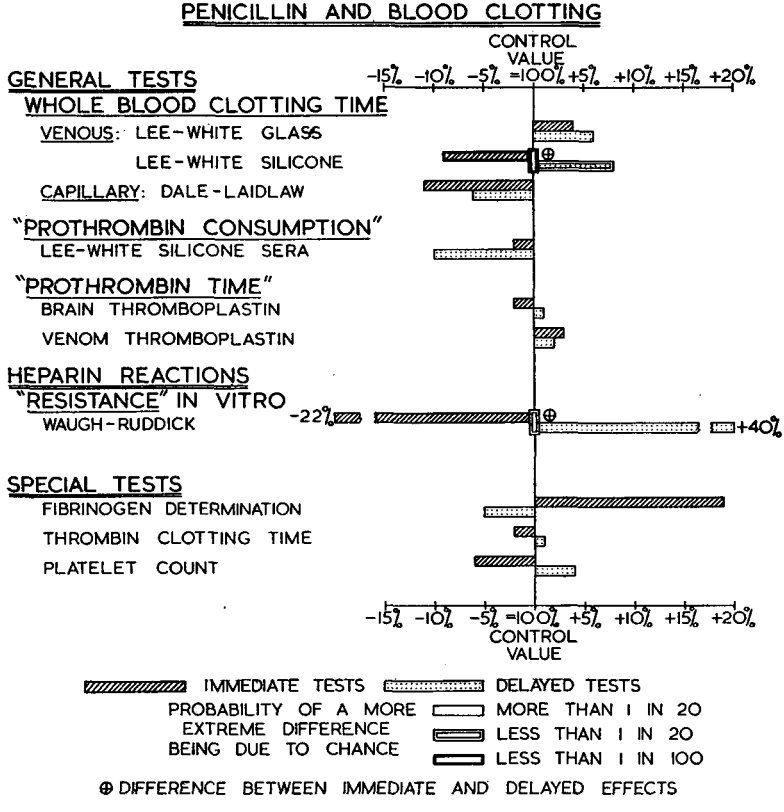

FIG. 1. *Experiment* 1: *Penicillin.* Magnitudes of differences between test and control results for each test with indications of statistical significance. The penicillin values are shown as the percentages by which they differ from the respective control values, which are taken as 100 per cent. For the de Takats test, see Fig. 2.

the clotting times of mixtures of 0·1 ml. of serum, fibrinogen solution, M/40 calcium chloride solution and acetone-dried human brain extract (Biggs and Macfarlane[7]). The mean was taken of 2 replicate readings in Experiment 1 and of 4 in Experiment 2. None of the "glass" sera clotting times was pathologically short. All the "silicone" sera clotting times were therefore accepted for analysis.

The plasma "prothrombin time" was measured by the one-stage technique with both human brain thromboplastin as above and with Russell's

viper venom, taking the mean of 2 to 4 replicate readings on each plasma sample.

The heparin "resistance" test (Waugh and Ruddick[8,9]) was made on citrated whole blood against 6 concentrations of heparin, a single series being tested with each sample. The results were summarised as the slope of the regression of clotting time on heparin concentration.

The heparin "tolerance" test (de Takats[10]) was made by giving an intravenous injection of heparin after the venepuncture and the Dale-Laidlaw tests; in Experiment 1, 500 U./stone of body-weight were given, and in Experiment 2, 5000 U. to each subject. Thereafter at intervals

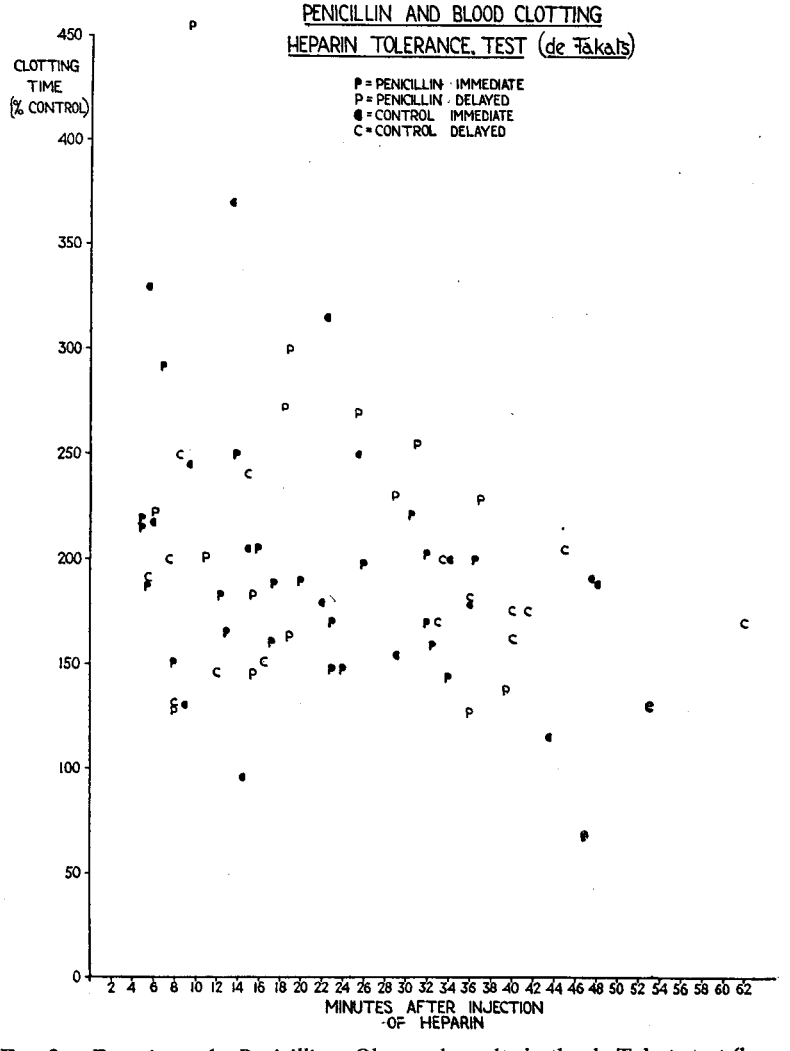

FIG. 2. *Experiment* 1: *Penicillin.* Observed results in the de Takats test (heparin "tolerance," *in vivo*) in control and penicillin subjects.

the Dale-Laidlaw clotting times were obtained from alternate ear lobes and were expressed as percentages of the mean Dale-Laidlaw clotting time before the injection of heparin. This test was abandoned in Experiment 2 because of the magnitude of the experimental error.

The plasma fibrinogen concentration was determined by a clot-weight method (Ingram[11]) on a single plasma sample at each testing.

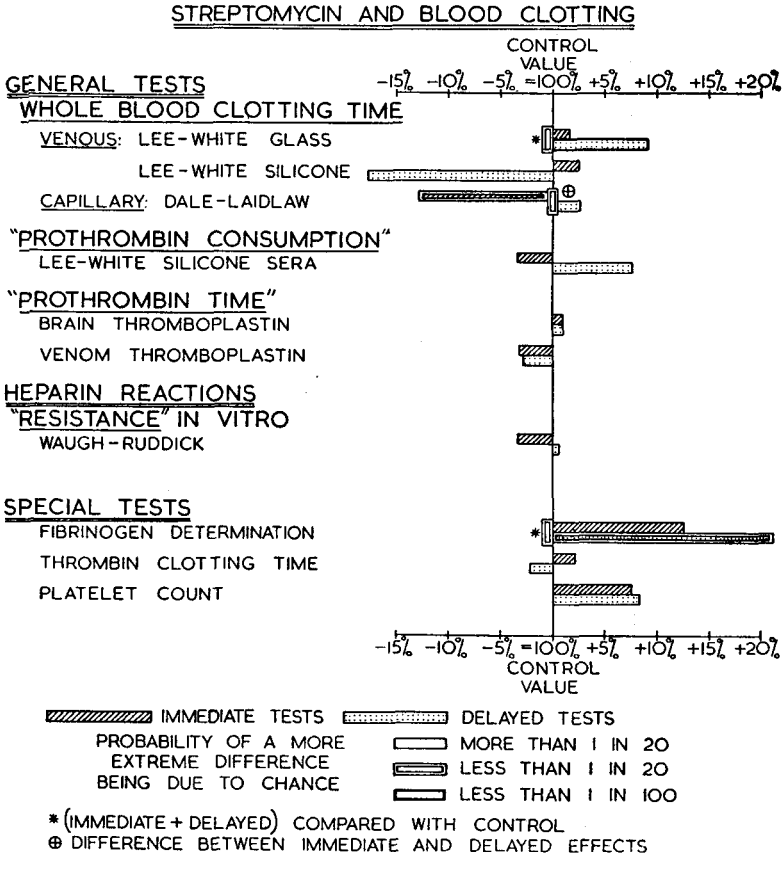

FIG. 3. *Experiment* 2: *Streptomycin.* Magnitudes of differences between test and control results for each test with indications of statistical significance. The streptomycin values are shown as the percentages by which they differ from the respective control values, which are taken as 100 per cent. For the de Takats test, see Fig. 4.

The thrombin clotting time was measured on citrated plasma with solutions of purified human thrombin (Lister Institute), the mean of 2 to 4 replicate tests being taken.

Platelet counts were made on capillary blood by the method of Baar.[12]

(ii) *Other tests. Serum drug concentrations* were estimated by the tube dilution method using the Oxford staphylococcus for penicillin and the Klebsiella K41 for streptomycin.

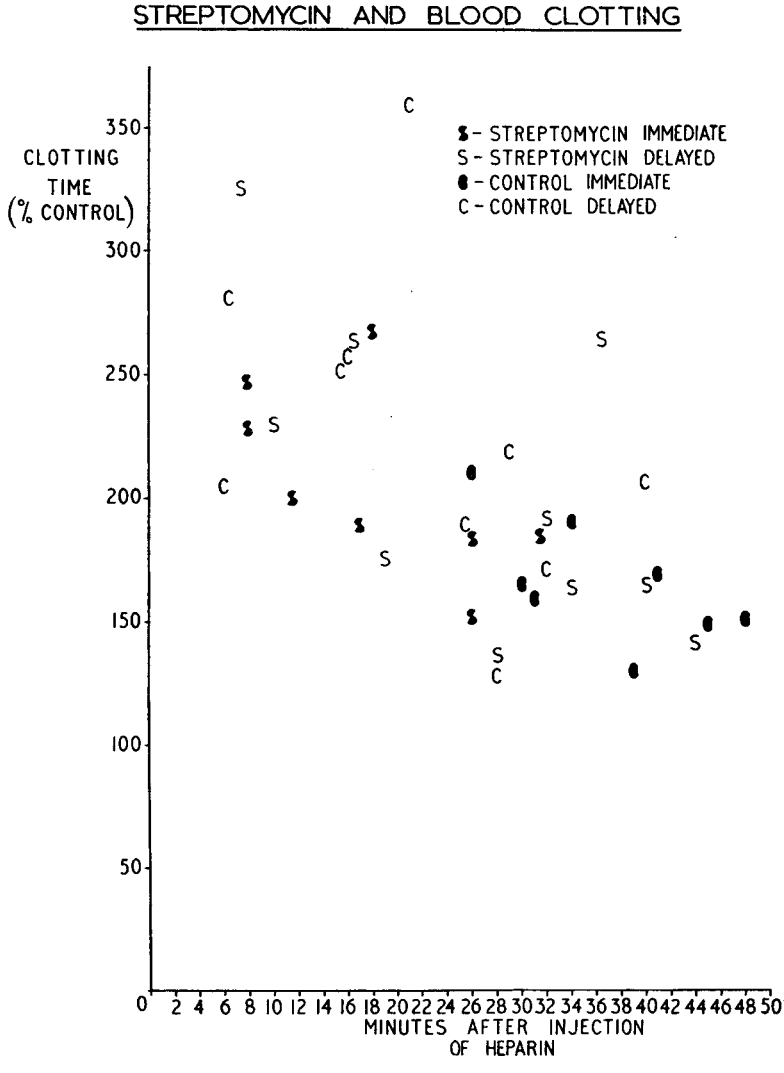

FIG. 4. *Experiment* 2: *Streptomycin.* Observed results in the de Takats test (heparin "tolerance," *in vivo*) in control and streptomycin subjects.

Plasma fats were estimated as it was thought that the random variation in the clotting tests might be reduced by taking into account the plasma concentrations of fatty substances (Waldron and Friedman[13]). The following techniques were used: *total fatty acids* by the method of Stewart and Hendry[14]; *total cholesterol* by the method of Sackett[15]; *free cholesterol* by the method of Schoenheimer and Sperry[16]; and the *lipoid phosphorus* by the method of Stewart and Hendry.[17]

2. *Experiment* 3

Drug Administration and Controls

2 groups each of 4 subjects were submitted to immediate tests only, testing all 4 subjects on each experimental day according to the scheme of Table III, and testing the 2 groups on different days. The subjects received on different days one of the following injections: 1·0 mega-unit of

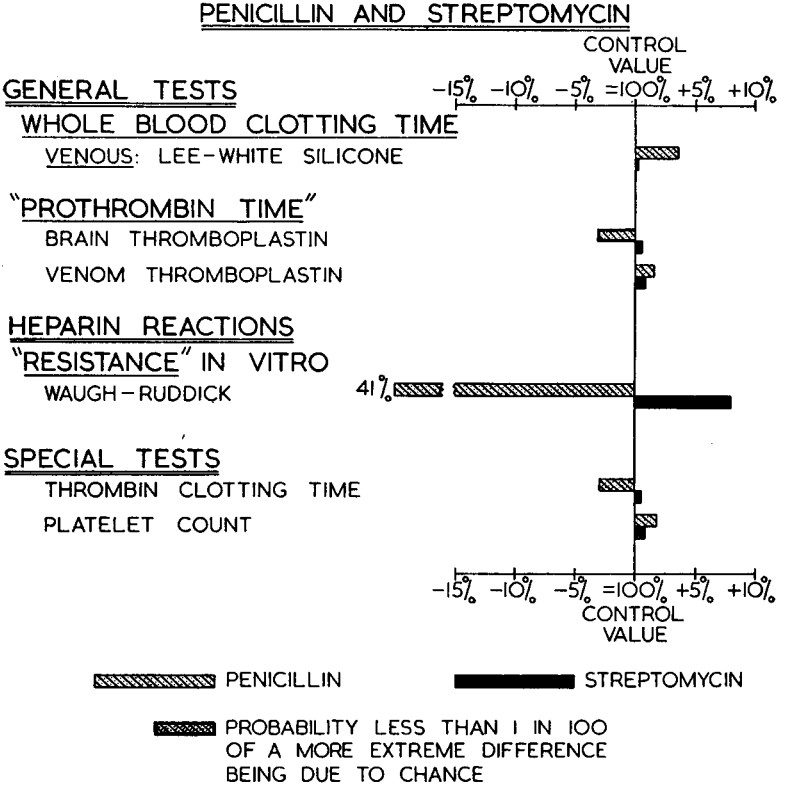

FIG. 5. *Experiment* 3: *Penicillin and streptomycin.* Magnitudes of differences between test and control results for each test (immediate effects only). The test results are shown as the percentages by which they differ from the respective control results, which are taken as 100 per cent.

crystalline sodium penicillin G; 2·0 g. of dihydrostreptomycin sulphate; 0·3 mg. of adrenaline tartrate (synthetic); and saline solution. The adrenaline and the saline solution were given as controls of emotional responses to the investigation: the subjects believed in fact that 4 different batches of penicillin were under investigation. Since they were not aware that they might receive adrenaline, and it was, thus, not desirable to make any systematic clinical observations for signs of adrenaline activity in the subjects concerned, indices of adrenaline activity were sought by platelet counting and by making blood sugar estimations.

TABLE III

GRAECO-LATIN ARRANGEMENT USED IN ALLOCATING INJECTIONS AND ORDER OF INJECTIONS IN EXPERIMENT 3

P = penicillin; S = streptomycin; a = adrenaline; s = saline

Suffix numerals determine the order of injecting the subjects on each day. The order of injection departs from the balanced arrangement required for a Graeco-Latin square but the other, more important, factors are perfectly balanced.

Group (i)					Group (ii)				
Week and day of test	Subjects				Week and day of test	Subjects			
	I	II	III	IV		V	VI	VII	VIII
Week 1. Monday	S_4	P_2	S_1	a_3	Week 1. Friday	s_4	P_3	S_1	a_2
Week 2. Monday	S_2	a_4	s_3	P_1	Week 2. Friday	S_2	a_4	s_3	P_1
Week 3. Friday	a_1	S_4	P_3	s_2	Week 3. Monday	a_1	S_4	P_2	s_3
Week 4. Friday	P_3	s_1	a_4	S_2	Week 4. Monday	P_3	s_1	a_2	S_4

Tests Used

(i) *Clotting tests.* The Lee-White silicone *whole blood clotting time* as above.

The plasma "prothrombin time" as above.

The heparin "resistance" test was made on slow-spun citrated plasma, following the modification of Silvermann[18] and testing 4 heparin concentrations. The results were summarised as before.

The thrombin clotting time as above.

The platelet count on venous blood, otherwise as above.

In the plasma prothrombin time and thrombin clotting time tests the samples from the 4 subjects were tested concurrently, 4 replicates being obtained from the venepuncture on each subject on each day. The 4 samples were also examined concurrently in the heparin "resistance" test, 1 reading being obtained from the venepuncture on each subject against each heparin concentration on each day. In all these tests, randomised Latin square arrangements were used to eliminate systematic errors on order of testing.

(ii) *Other tests. Serum concentrations of penicillin and streptomycin* were determined as above.

The blood sugar was determined on oxalated blood by the method of Hagedorn and Jensen.[19]

RESULTS

Presentation of Results

Experiments 1 and 2 each provided about 1000 individual readings and Experiment 3 about 400. Raw data are therefore not usually given but the summarised data are arranged principally to indicate the magnitude and significance of the observed differences between mean drug and control results. The standard errors of the differences were obtained from analyses of variance in which overall differences between subjects and between days have been eliminated from the treatment comparisons.

1. *Serum concentrations of penicillin and streptomycin.* Table IV shows the serum concentrations of penicillin and of streptomycin obtained in the 3 experiments. The accuracy of the determinations is subject to the usual limitations of twofold dilution assays.

2. *Adrenaline activity.* Table V shows the mean platelet counts and the mean blood sugar concentrations observed in the adrenaline and saline subjects in Experiment 3. It appears that in neither test was the dose of adrenaline sufficient to evoke a response. As significant differences are not apparent in the clotting data from these two groups, this material has therefore been pooled in the subsequent analysis, with the exception of the "prothrombin time" test with brain thromboplastin as shown in Table VIIIB.

TABLE IV

SERUM CONCENTRATIONS OF PENICILLIN AND OF STREPTOMYCIN OBTAINED IN THE DRUG SUBJECTS IN THE THREE EXPERIMENTS

Except at the extreme concentrations, the results are expressed as the mean of the tested concentrations just permitting and just inhibiting the growth of the test organism. The entries are derived from the 6, 6 and 8 subjects respectively

Experiment 1 Penicillin I.U./ml.		Experiment 2 Streptomycin I.U./ml.		Experiment 3	
				Penicillin (immediate) I.U./ml.	Streptomycin (immediate) I.U./ml.
Immediate	Delayed	Immediate	Delayed		
7·5	0·03	80	10	20	20
15	0·06	40	10	15	40
15	0·03	20	2·5	30	40
4	0·03	5	2·5	15	20
15	0·03	20	10	15	10
7·5	0·1	40	2·5	0·06*	1·2*
				30	20
				20	15

* These low values occurred in different subjects on the same day. In both cases a quantity of the drug was lost at the time of injection.

TABLE V

PLATELET COUNTS AND BLOOD SUGAR CONCENTRATIONS OBSERVED IN ADRENALINE AND SALINE SUBJECTS IN EXPERIMENT 3

8 observations were available in each case

S.E. = Standard error of the difference between the means
P = Probability of obtaining, by chance, a difference more extreme than that observed

Test	Adrenaline subjects		Saline subjects		Difference ± S.E. between means, with significance
	Mean	(Range)	Mean	(Range)	
Platelet count thousands/cu. mm.	320	(261–390)	331	(262–390)	-11 ± 15 $(0{\cdot}4 < P < 0{\cdot}5)$
Blood sugar mg. per cent. ..	96·8	(87–112)	94·5	(69–147)	$2{\cdot}3 \pm 4{\cdot}8$ $(0{\cdot}6 < P < 0{\cdot}7)$

3. *Effects due to penicillin and to streptomycin.* The analyses of the results of the 3 experiments are given in Tables VI-VIIIA and B. A number of the drug-control differences are shown as statistically significant. The significance is probably spurious in the immediate Lee-White silicone test of Experiment 1 and in the delayed Lee-White glass test of Experiment 2, because in these cases the estimate of residual variation used in the analysis of variance is, by chance, actually smaller than its error component.

(The estimates of the total residual variances, derived from the analyses of variance, are based on at most 7 degrees of freedom in Experiments 1 and 2, and 12 in Experiment 3. For each test a separate estimate of the

experimental error, which is one component of the total variance, can be obtained from the scatter between replicates, and, being based on a large number of degrees of freedom, is relatively more accurate. These error components were therefore always estimated as a check on the residual variances, whenever replicate readings were obtained.)

The magnitudes of drug-control differences are shown diagrammatically in Figures 1, 3 and 5, except for the de Takats tests, of which the actual data are shown as scatter diagrams in Figures 2 and 4. Treatment effects

TABLE VI

EXPERIMENT 1. THE EFFECTS OF PENICILLIN

Test	Immediate observations			Delayed observations		
	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.
1. Lee-White (glass) (minutes) ..	13·08	12·58	+0·50 ± 0·72	12·78	12·02	+0·76 ± 0·72
2.‡Lee-White (silicone (minutes) ..	39·13	43·13	−4·00** ± 0·88	39·05	36·32	+2·73* ± 0·88
3. Dale-Laidlaw (minutes) ..	2·14	2·41	−0·27 ± 0·18	2·19	2·34	−0·15 ± 0·18
4. "Prothrombin consumption" (seconds) ..	21·5	22·0	−0·5 ± 1·6	21·8	23·9	−2·1 ± 1·6
5. "Prothrombin time" (brain thromboplastin) (seconds) ..	15·30	15·65	−0·35 ± 0·22	16·01	15·85	+0·16 ± 0·22
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	15·17	14·68	+0·49 ± 0·37	13·80	13·50	+0·30 ± 0·37
7. Thrombin clotting time (seconds) ..	7·66	7·82	−0·16 ± 0·13	7·00	6·93	+0·07 ± 0·13
8.†Waugh-Ruddick (heparin) (minutes/u. heparin)‖ ..	34·0	43·6	−9·6 ± 7·1	50·9	36·5	+14·4 ± 7·1
9. Platelet count (thousands/cu. mm.)	316	335	−19 ± 30·0	399	384	+15 ± 30
10. Fibrinogen concentration (g. per cent.) ..	0·305	0·256	+0·049 ± 0·028	0·254	0·266	−0·012 ± 0·028

* Different significant at the 5 per cent. level.
** Different significant at the 1 per cent. level.
† Difference between immediate and delayed drug values significant at the 5 per cent. level.
‡ Difference between immediate and delayed drug values significant at the 1 per cent. level.
‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 7 degrees of freedom for error: where $n = 7$, $t = 2{\cdot}365$ for $P = 0{\cdot}05$.

in the Lee-White whole blood clotting times were also non-significant when studied as the ratio of the silicone time to the glass time.

4. *Correlation between clotting times and blood fats* (*Experiments* 1 *and* 2). It was thought that if a significant correlation could be demonstrated between clotting times and blood fats the effect would be most obvious in the Lee-White silicone whole blood clotting time and in the venom "prothrombin time." In neither case was a significant correlation found and thus no reduction in random error could be obtained. All the fat values were thought to be within normal limits for the several determinations.

TABLE VII

EXPERIMENT 2. THE EFFECTS OF STREPTOMYCIN

Test	Immediate observations			Delayed observations		
	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.
1. Lee-White (glass) (minutes) ..	13·02	12·82	+0·20 ± 0·30	13·23	12·11	+1·12** ± 0·30
2. Lee-White (silicone) (minutes) ..	32·10	31·32	+0·78 ± 5·05	28·78	34·98	−6·20 ± 5·05
3.†Dale-Laidlaw (minutes) ..	1·66	1·90	−0·24* ± 0·08	1·77	1·73	+0·04 ± 0·08
4. "Prothrombin consumption" (seconds) ..	21·8	22·6	−0·8 ± 3·2	18·4	17·1	+1·3 ± 3·2
5. "Prothrombin time" (brain thromboplastin) (seconds) ..	14·12	13·98	+0·14 ± 0·19	13·98	13·85	+0·13 ± 0·19
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	14·17	14·63	−0·46 ± 0·73	14·99	15·44	−0·45 ± 0·73
7. Thrombin clotting time (seconds) ..	10·68	10·47	+0·21 ± 0·16	9·40	9·60	−0·20 ± 0·16
8.‖Waugh-Ruddick (heparin) (minutes/u. heparin) ..	24·5	25·4	−0·9 ± 5·8	18·7	18·6	+0·1 ± 5·8
9. Platelet count (thousands/cu. mm.)	328	305	+23 ± 24·0	288	266	+22 ± 24
10. Fibrinogen concentration (g. per cent.) ..	0·340	0·308	+0·032 ± 0·017	0·394	0·325	+0·069* ± 0·017

* Difference significant at the 5 per cent. level.
** Difference significant at the 1 per cent. level.
† Difference between immediate and delayed drug values significant at the 5 per cent. level.
‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only seven degrees of freedom for error: where $n = 7$, $t = 2{\cdot}365$ for $P = 0{\cdot}05$.

TABLE VIIIA

EXPERIMENT 3. THE EFFECTS OF PENICILLIN AND OF STREPTOMYCIN

Test	Pooled means for adrenaline and saline tests (= control)	Penicillin tests (immediate)		Streptomycin tests (immediate)	
		Mean	Difference between drug and control means ± S.E.	Mean	Difference between drug and control means ± S.E.
2. Lee-White (silicone) (minutes)	22·25	23·05	+0·80 ± 1·36	22·30	+0·05 ± 1·36
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	14·87	15·11	+0·24 ± 0·20	15·00	+0·13 ± 0·20
7. Thrombin clotting time (seconds)	16·19	15·68	−0·51 ± 0·50	16·40	+0·21 ± 0·50
8. Waugh-Ruddick (heparin) (minutes/u. heparin)‖	60·00	35·10	−24·90 ± 13·05	64·80	+4·80 ± 13·05
9. Platelet count (thousands/cu. mm.) ..	325	331	+6 ± 13	328	+3 ± 13

The pooled means for the adrenaline and saline tests have been used as control values. No differences are significant.

‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 12 degrees of freedom for error: where $n = 12$, $t = 2{\cdot}179$ for $P = 0{\cdot}05$.

5. *Differences between subjects and between days.* A number of significant differences were detected between mean results obtained from different subjects and on different days, but the design of these experiments was not suitable for studying the magnitudes of these differences and they are therefore not presented.

TABLE VIIIB

EXPERIMENT 3. THE EFFECTS OF PENICILLIN AND OF STREPTOMYCIN

Mean clotting times (seconds) for the brain "prothrombin time", to show significant differences.

Controls			Penicillin tests (immediate)		Streptomycin tests (immediate)	
Saline	Adrenaline					
Mean	Mean	Difference between drug and saline means ± S.E.	Mean	Difference between drug and saline means ± S.E.	Mean	Difference between drug and saline means ± S.E.
15·31	14·64	−0·67* ± 0·24	14·56	−0·75** ± 0·24	14·94	−0·37 ± 0·24

* Difference significant at the 5 per cent. level.

** Difference significant at the 1 per cent. level.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 12 degrees of freedom for error: where $n = 12$, $t = 2{\cdot}179$ for $P = 0{\cdot}05$.

6. *Residual error variances: the experimental error of the tests.* In tests where replicate readings have been obtained, the error component of the residual variance can be directly determined, and the experimental error of the different tests may be usefully compared by means of the coefficient of variation. These data are shown in Table IX.

DISCUSSION

1. *Effects due to Penicillin and to Streptomycin*

(i) *Clotting time tests.* In certain tests the clotting times appear to have been influenced by penicillin and by streptomycin, but the irregular

TABLE IX

EXPERIMENTAL ERRORS: Approximate values for the standard deviations for experimental error have been obtained from the ranges observed within replicates: for purposes of comparison these values have been expressed as percentages of the corresponding means

Test	Coefficients of variation per cent. (from scatter within replicates)				
	Experiment 1 (penicillin)	Experiment 2 (streptomycin)	Experiment 3 (both drugs)	Unweighted mean (present 3 experiments)	Estimates of Briggs and MacMillan (1948)
1. Lee-White (glass)	14·7	11·3		13·0	7·0
2. Lee-White (silicone)	15·9	20·2	13·0	16·4	
3. Dale-Laidlaw	9·3	10·8		10·1	25·1
4. "Prothrombin consumption"	7·0	7·8		7·4	
5. "Prothrombin time" (brain thromboplastin)	2·0	4·4	5·2*	3·9	
6. "Prothrombin time" (venom thromboplastin)	3·0	2·6*	1·7	2·4	
7. Thrombin clotting time	3·7	4·9	6·5*	5·0	

* In these tests a different observer recorded the results in the indicated instances: in the other tests observations were mostly obtained by the same observer(s).

pattern of the significant effects is bizarre. Moreover, individual significant effects were of the order of only a 10 per cent. increase or decrease over control values. It therefore seems wise to ascribe no fundamental importance to any of these effects: where a number of tests are made an occasional "significant" effect of low magnitude must be expected to arise by chance. Hence this work does not support the earlier claims of a coagulant effect of penicillin and of streptomycin of which Macht[20,21,22,23] has been the chief exponent.

Macht's (*passim*) studies are difficult to discuss. There is no doubt of the marked effects on clotting time of these and many other drugs apparent in his published data. Nevertheless his results may fairly be questioned in the many instances in which he has not used concurrent controls but has compared the clotting times obtained after the administration of a drug with those previously obtained from the same subject. In such circumstances it is always possible that the shortening in clotting time is due to some other cause, such as the progress of anæsthesia, the effect of restraint in the unanæsthetised animal, the method of administration of the drug or even to the effect of obtaining serial blood samples. For instance, a shortening of the clotting time following emotional reaction in the cat was demonstrated by Cannon and Mendenhall[24] and a similar effect in animals and in man has recently been studied by Macht[25] himself. Menghini and Giunti[26] have observed in man a progressive shortening of clotting time in serial venepunctures without the administration of any drug.

(ii) *Plasma fibrinogen determination.* This measurement is probably liable to less subjective error than the observations of clotting time. It is therefore reasonable to look for a biological explanation of the 20 per cent. rise in the delayed streptomycin value over control, significant at the 5 per cent. level, in Experiment 2 (Table VII). This might be due to a mild toxic action of the drug: a rise in the plasma fibrinogen concentration is a sensitive indication of toxic or inflammatory processes (Foster and Whipple[27]; Gram[28]; Schultz *et al.*[29]) and the body might respond in this way to streptomycin before clinical evidence of toxicity was apparent. A subclinical toxic action of streptomycin has also been detected electrocardiographically by di Maria.[30]

2. *Residual Error Variances*

Table IX shows great differences between the error coefficients of the various tests. The heparin resistance test showed the greatest variability and the plasma tests the least.

Biggs and MacMillan[31] obtained estimates of experimental error for whole blood clotting times (Lee-White glass and Dale-Laidlaw) in their detailed study of laboratory errors. Their estimates are based on differences between replicates, with no interaction; their values are included in Table IX for comparison.

SUMMARY

1. The effects of penicillin and of streptomycin on blood coagulation have been studied on 20 normal subjects after injection of the drugs.

Each subject acted in turn as "test" and "control" and 3 experiments were made: 2, each using 6 subjects, on the immediate and delayed effects of the 2 drugs separately, and 1, using 8 subjects, on the immediate effects of both drugs. Symmetrical experimental designs eliminated differences between subjects and between days of test. 9 tests of clotting function were used, in addition to the counting of platelets and the determination of plasma fibrinogen.

2. A few significant differences were detected between test and control subjects but no systematic effect was apparent through all 3 experiments.

3. Estimates are given for the experimental error of the various tests. The error was relatively low for one-stage "prothrombin times" and thrombin clotting times, and relatively high for whole-blood-clotting times.

4. No support has been obtained for previous work reporting a coagulant action of penicillin and streptomycin.

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