From the available evidence the most likely cause of the anaemia is an alteration of the intestinal flora analogous to that which occurs in cases of gastro-colic fistula in man, and it is possible that the intestinal bacteria are in some way concerned with the absorption or utilization of haemopoietic substances.

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Nearly 800 new members were recruited in England and Wales to the National Hospital Service Reserve in November, bringing the total strength to 18,573. Of these, 1,706 are trained nurses and 16,839 nursing auxiliaries. Newcastle Hospital Region headed the November recruiting with 200 members, more than a quarter of the month's total of 796. Manchester came second with 102, and Bristol third with 94. Wales still has the largest total membership, 3,634, followed by the South-west Metropolitan Area with 2,277, and Newcastle with 1,643. A new recruiting drive was launched at the end of November by Miss Hornsby-Smith, Parliamentary Secretary to the Ministry of Health, with the aim of trebling membership and so bringing the Reserve well on the way to its peacetime objective of 80,000 members.

THE EFFECT ON BLEEDING-TIME OF AN EXTRACT OF BLOOD OF PATIENTS WITH RHEUMATOID ARTHRITIS

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It has for many years been a matter of discussion among those interested in the origins of rheumatoid arthritis that the disease may arise in apparent association with a large number of disturbing incidents, including infection, trauma, burns, excessive fatigue, the use of certain drugs, the metabolic crises of puberty, child-bearing, and the climacteric, and the psychological stresses and strains of normal life. That these incidents were the only cause was never believed, and statisticians who disproved to the hilt their sole significance might have excused themselves the labour of belabouring a donkey which had never lived.

Nevertheless, to experienced clinicians it was obvious that, though the majority of people could suffer with impunity the "slings and arrows of outrageous fortune," there were others who could not. Their interest was centred not on the hammer's stroke but on the anvil's ring, and was intensified by the description by Hans Selye (1936b, 1937) of the alarm reaction, which concentrated their attention upon the adrenal cortex. From the beginning it seemed likely, on clinical grounds, that the adrenals, if involved at all in the story of rheumatoid arthritis, would be found to be normal in this condition, for no evidence of adrenal deficiency had been observed in extensive investigations of the biochemistry of rheumatism. It seemed more likely that the error would be found in a mechanism in which the adrenal cortex served as an essential part, and that it would be in the control of the adrenals or in the response to the adrenals that the error would be found.

The Literature

Various Soviet authors (Synovich, 1940; Pshenichnikov, 1940; Novikov, 1941—reviewed by Clarke, 1941) claimed to have shown that the systemic use of local anaesthetics had resulted in a significant decrease in traumatic shock. Their results were confirmed by Ungar (1943), who found that "nupercaine" (cinchocaine) was especially effective. Extending his observations, Ungar (1944) found that several procedures were capable of protecting guinea-pigs and rats against subsequent trauma by causing an inhibition of histamine release. These procedures included the production of previous minor trauma and the administration of ascorbic acid, nupercaine, procaine, cocaine, adrenaline in unphysiological doses, a whole cortical extract prepared by Kendall, anterior pituitary extract, and, pre-eminently, adrenocorticotrophic hormone (A.C.T.H.). The protection afforded by these procedures (except the

administration of ascorbic acid) could be passively transferred in the serum from one animal to another, and was therefore due to a substance contained in the serum.

Inhibition of histamine release and consequent protection could be obtained, however, in adrenalectomized rats only by ascorbic acid and by adrenal extracts. Moreover, the inhibitory substance could not be demonstrated in the blood of hypophysectomized animals. Ungar concluded that certain procedures, including trauma, and the injection of peptone and certain drugs, causes the pituitary to release into the circulation a substance which stimulates the adrenals, which in their turn initiate the production of a protective substance. His results afforded a possible explanation of the observation of many workers that hypophysectomized and adrenalectomized animals are highly sensitive to trauma, and of the fact that the former may be protected by the administration of A.C.T.H. (Reiss, Macleod, and Golla, 1943). Ungar considered that the mechanism he postulated was a part of the alarm reaction described by Selye.

Action of Splenic Extracts

In 1945 Ungar published a further communication in which he claimed to show that the protective substance in question is derived from the spleen, from which it is released under the stimulation of the adrenal cortex. He had previously shown that substances which inhibited histamine release reduced bleeding-time and increased capillary resistance. He selected reduction of bleeding-time as the easiest and most reliable test for the substances involved in the endocrine reaction to trauma, and found that a reduction occurred after trauma in normal rats but not in hypophysectomized rats unless these were treated with A.C.T.H., adrenal extract, or splenic extract. Secondly, the reduction occurred in

adrenalectomized rats only if they were treated with adrenal extract or splenic extract. Thirdly, the reduction occurred in splenectomized rats only if they were treated with splenic extract. Similar effects could not be induced with other tissue extracts.

A curious point about the action of some splenic extracts now came to light. Small doses reduced the bleeding-time, but large doses increased it. This anomaly was subsequently found to be due to the presence in the extracts of two substances named by Ungar "splenin A" and "splenin B" (Ungar, 1945, 1946-7), whose pharmacological effects are opposed to one another. "Splenin A" is probably a derivative of ascorbic acid (Ungar, 1946-7), and "splenin B" is an ester of two or three fatty acids with a complex alcohol (Ungar and Damgaard, 1951). Ungar succeeded in demonstrating "splenin B" in human serum in various pathological conditions (purpura, haemolytic jaundice, paroxysmal haemoglobinuria, scurvy, and, at our instigation, rheumatoid arthritis) and from the serum of guinea-pigs after thyroidectomy. We have found it (or a similar substance) in the serum in cases of thyrotoxicosis and rheumatoid arthritis (Greene, 1950), but never in that of healthy persons.

The spleen has been for many years an organ of special interest to students of the rheumatic diseases because of its enlargement in Still's disease and Felty's syndrome. The possible role of the adrenal cortex had attracted a curiously limited attention since the publications of Selye (1936a) had begun. The work of Ungar suggested a possible line of attack, and in 1946 he was kind enough to test a number of unlabelled samples of serum sent to him from the Metropolitan Hospital. Two samples from cases of rheumatoid arthritis contained no "splenin A" but appreciable quantities of "splenin B." Four samples from healthy blood donors contained "splenin A" but no "splenin B." The serum from the

TABLE I.—Data from Experiment 1 (Saline and Procaine)

Week	Guinea-pig	Group I				Group II			
		Initial Bleeding-time of Guinea-pigs (Seconds)				Responding Bleeding-time 20 mins. after 0.4 ml. 10% Procaine S.C. (Seconds)			
1	B1	115	120	130	120	80	85	95	90
	B2	115	135	130	125	85	75	120	90
	B3	125	170	140	140	120	95	110	85
		1,565				1,130			
2	A1	115	160	110	100	110	80	90	90
	A2	130	145	180	150	90	110	135	110
	A3	140	115	130	140	100	105	75	75
		1,615				1,170			
3	B1	135	135	150	150	130	85	120	90
	B2	140	115	125	130	100	95	120	75
	B3	130	140	100	130	80	105	70	130
		1,580				1,200			
4	A1	150	170	120	110	100	105	125	125
	A2	150	120	115	140	130	60	120	100
	A3	115	130	120	100	70	95	95	85
		1,540				1,210			
5	B1	170	160	150	100	140	90	140	110
	B2	160	100	115	130	95	75	90	90
	B3	145	120	110	160	140	70	105	90
		1,620				1,235			
6	A1	120	120	130	170	85	85	85	120
	A2	140	130	110	160	120	90	105	70
	A3	120	140	130	110	90	105	65	110
		1,580				1,130			
Grand Totals		9,500				7,075			
Average Time		132 seconds				98 seconds			
	B1	140	125	100	115	135	115	130	105
	B2	165	115	105	130	105	145	115	125
	B3	155	130	125	120	160	125	95	120
		1,525				1,475			
	B1	125	145	165	115	160	100	130	105
	B2	95	135	95	135	90	110	130	155
	B3	130	155	120	120	125	120	155	95
		1,535				1,455			
	A1	120	95	140	120	150	90	160	130
	A2	130	110	125	95	130	150	130	135
	A3	160	110	120	145	95	90	150	130
		1,470				1,540			
	B1	170	140	130	150	165	125	100	155
	B2	130	90	125	135	165	125	115	150
	B3	130	155	120	130	90	160	120	100
		1,605				1,570			
	A1	130	110	100	100	115	130	90	125
	A2	160	140	150	130	135	180	150	115
	A3	160	120	110	100	130	120	130	170
		1,510				1,590			
	B1	160	125	145	100	130	110	130	120
	B2	140	95	120	100	120	110	140	130
	B3	165	125	120	80	140	90	150	125
		1,475				1,495			
Grand Totals		9,120				9,125			
Average Time		127 seconds				127 seconds			

rheumatic patients was tested again after clinically successful treatment with A.C.T.H. and found to contain "splenin A" but no "splenin B" (Greene, 1950).

These results were encouraging, but it was clear that they might be fortuitous. The hypothesis advanced by Ungar rested on a foundation which seemed insufficiently solid—the estimation of bleeding-time in a comparatively small number of guinea-pigs. It was therefore decided to submit his method to a careful experimental trial.

Experiment 1

The first experiments were carried out to find the variation of the normal bleeding-time of guinea-pigs under carefully controlled conditions and the effect on it of a single dose of procaine. The bleeding-time was measured on the ear. Four readings were made, two on each ear, and the average of these was taken as the mean bleeding-time. The ear was pricked with a needle (1 mm. in diameter) and the bleeding observed and the wound blotted at intervals of five seconds. Bleeding was considered to have stopped when no issue of blood or serum could be detected for 10 seconds (Ungar, 1945). Six albino guinea-pigs of the same sex and approximately equal weight were chosen for the experiment. Each week the average bleeding time was measured on three of the animals before and 20 minutes after an injection of 0.4 ml. of 10% procaine, and, as a control, on three before and 20 minutes after an injection of 0.4 ml. of normal saline. This was continued for six weeks, so that both sets of guinea-pigs had alternate injections of procaine and saline. All injections were given subcutaneously. The results are recorded in Table I.

FIG. 1.—Histogram of normal guinea-pigs.

FIG. 2.—Histogram of guinea-pigs after saline injection (—) and after procaine injection (----).

The figures in Table I are expressed as histograms (Figs. 1 and 2). The first shows the variation in bleeding-time of normal guinea-pigs. Out of 144 measurements 77% lie between 100 and 155, with an average of 129 seconds. The form of the distribution is symmetrical and approximately normal. Fig. 2 shows that the measurements after saline injection are fairly similar. Out of 72 observations 76% lie between 100 and 155, with an average of 132 seconds. In contrast, the measurements after procaine injection are very different. Of the observations 79% lie between 75 and 135, with an average of 98 seconds. The histograms in Fig. 2 overlap to the extent of 33%. It is accordingly desirable to have a more sensitive test for a real difference than one based only on the appearance of the histograms.

The data from Table I give the variance analysis shown in Table II, demonstrating that each of the six guinea-pigs has a characteristic bleeding-time (variance

TABLE II.—Analysis of Variance (Experiment 1)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Variation due to characteristic bleeding-times of each guinea-pig ..	5,860	5	1,172	2.87	0.05 to 0.01
Variation due to characteristic day effects on the bleeding-time ..	1,369	5	274	0.67	N.S.*
Interaction of the above effects with each other	5,683	10	568	1.39	N.S.
Effect of procaine compound with all other groups ..	49,202	1	49,202	120.59	0.001 >
Variation among other groups—e.g., normal groups and saline group	1,320	2	660	1.62	N.S.
Residue remaining after removal of the above effects regarded as error arising randomly† ..	107,731	264	408	—	—
Total variation from all sources ..	171,165	287	—	—	—

* N.S. signifies non-significant in the sense that the differences found are no greater than would be expected to occur by chance. Significance is conventionally regarded as beginning at the once-in-twenty-times level.

† The residue was extensively examined in order to observe other systematic effects. None were found, and it was therefore expressed as a single item.

ratio=2.87, $n_1=5$, $n_2=264$, $0.05 > P > 0.01$), which persists from day to day (variance ratio=1.39, $n_1=10$, $n_2=264$, not significant). On all six days of the experiment the bleeding-times appear not to have changed (variance ratio=0.67, $n_1=5$, $n_2=264$, not significant). The normal bleeding-times of guinea-pigs do not differ from the bleeding-times after saline injection (variance ratio=1.62, $n_1=2$, $n_2=264$, not significant). The procaine-treated group differ considerably (variance ratio=120.59, $n_1=1$, $n_2=264$, $P=0.001$)—more, in fact, than would be supposed from the histograms, since here the bias of each individual guinea-pig has been eliminated. Clearly, procaine has been effective in reducing the bleeding-time.

Experiment 2

The second set of experiments was designed to compare the effects of serum from normal subjects and from patients suffering from active rheumatoid arthritis, all of whom had an E.S.R. of over 20 mm. in the first hour, measured by Wintrobe's method. In each case the serum was collected, pooled, and freeze-dried in 20-ml. portions and kept until required.

The serum was extracted with chloroform and acetone in order to separate Ungar's chloroform-soluble "splenin A" from the acetone-soluble "splenin

B." For each test 20 ml. of serum was shaken with three 20-ml. portions of chloroform. The combined chloroform extracts were filtered and evaporated to dryness, leaving a residue of "splenin A" and "B." To separate the "splenin B" from the residue, 20 ml. of acetone was added. The acetone was then decanted and evaporated, leaving the dry extract of "splenin

B." Just before testing, 2 ml. of saline was added to both residues and 1 ml. per kg. used for injection.

In each case the average bleeding-times of the guinea-pigs were recorded every week, and the day before and one hour after injection. The experiment was continued for twelve weeks, the same guinea-pigs being used as in the previous experiment.

TABLE III.—Data from Experiment 2 (Normal Serum)

Week	Group III								Group IV									
	Guinea-pig	Initial Bleeding-time of Guinea-pigs				Responding Bleeding-time 1 Hour after Extract A S.C.				Guinea-pig	Initial Bleeding-time of Guinea-pigs				Responding Bleeding-time 1 Hour after Extract B S.C.			
1	C1	145	110	130	120	120	120	75	65	D1	130	180	125	100	110	135	125	170
	C2	90	130	100	120	110	80	95	95	D2	150	95	165	105	160	120	170	120
	C3	95	140	120	120	110	100	105	100	D3	125	120	120	140	120	100	120	160
		1,420				1,175					1,555				1,610			
2	D1	110	170	120	75	100	120	100	75	C1	160	95	95	175	119	140	90	100
	D2	120	155	100	95	150	150	100	70	C2	110	110	105	130	100	125	125	105
	D3	90	130	165	90	90	90	95	80	C3	160	120	180	100	125	130	130	140
		1,420				1,220					1,540				1,429			
3	C1	120	95	100	180	70	115	135	110	D1	100	100	120	150	130	135	160	85
	C2	110	100	110	90	70	90	85	120	D2	180	130	150	70	180	90	115	125
	C3	140	125	180	100	125	120	110	95	D3	125	120	175	175	180	130	135	100
		1,450				1,245					1,595				1,565			
4	D1	140	90	120	105	125	75	60	80	C1	140	100	130	130	125	110	100	140
	D2	165	105	150	90	85	60	90	150	C2	120	140	130	120	80	130	100	160
	D3	145	120	125	110	140	80	95	100	C3	120	120	105	105	95	125	110	165
		1,465				1,140					1,460				1,440			
5	C1	170	85	120	120	90	105	95	125	D1	140	160	125	110	170	70	120	65
	C2	95	135	120	180	125	145	70	105	D2	180	100	105	135	165	90	65	120
	C3	110	150	90	110	130	155	70	170	D3	180	130	140	95	115	160	90	120
		1,485				1,385					1,600				1,350			
6	D1	145	115	125	150	140	115	125	70	C1	100	150	140	100	90	110	170	105
	D2	120	130	175	80	60	100	85	95	C2	130	95	105	140	80	110	140	150
	D3	100	110	135	115	75	135	90	75	C3	140	100	135	110	160	70	105	130
		1,500				1,165					1,445				1,420			
Grand Totals		8,740				7,330					9,195				8,814			
Average		122 seconds				102 seconds					128 seconds				123 seconds			

TABLE IV.—Data from Experiment 2 (Rheumatoid Serum)

Week	Group V								Group VI									
	Guinea-pig	Initial Bleeding-time of Guinea-pigs				Responding Bleeding-time 1 Hour after Extract A				Guinea-pig	Initial Bleeding-time of Guinea-pigs				Responding Bleeding-time 1 Hour after Extract B			
7	E1	125	105	180	120	70	75	140	155	F1	100	125	135	110	200	90	90	175
	E2	110	130	95	160	145	95	100	145	F2	165	110	95	105	125	195	190	140
	E3	120	100	155	100	95	120	145	120	F3	185	135	120	90	210	120	140	135
		1,500				1,405					1,475				1,810			
8	F1	120	130	180	135	180	180	60	90	E1	175	120	135	180	120	125	160	120
	F2	80	130	120	130	155	100	200	60	E2	95	185	145	125	230	180	120	210
	F3	120	175	120	120	100	100	90	120	E3	135	110	175	90	145	165	165	160
		1,560				1,435					1,670				1,900			
9	E1	120	135	105	140	135	110	170	70	F1	135	80	150	135	150	145	180	120
	E2	160	125	85	180	95	110	170	90	F2	130	110	140	135	160	120	140	240
	E3	90	105	180	145	170	110	110	110	F3	120	135	185	95	165	100	180	120
		1,570				1,450					1,550				1,820			
10	F1	96	109	80	100	60	164	118	146	E1	135	134	108	137	157	117	212	150
	F2	118	119	130	145	115	157	125	105	E2	110	120	150	120	120	130	180	215
	F3	150	60	135	108	145	87	95	140	E3	120	145	87	136	145	125	165	166
		1,350				1,457					1,502				1,882			
11	E1	112	120	105	127	180	109	143	135	F1	125	115	95	85	190	120	150	186
	E2	100	110	150	155	150	95	90	96	F2	135	140	180	110	131	125	215	100
	E3	145	100	128	95	150	88	124	126	F3	124	160	151	125	138	219	90	135
		1,447				1,486					1,545				1,799			
12	F1	96	90	125	100	92	66	120	166	E1	123	101	150	160	135	100	154	131
	F2	90	105	130	143	130	78	155	109	E2	90	120	125	135	162	173	103	180
	F3	100	134	120	100	120	102	150	87	E3	111	197	120	95	118	130	144	120
		1,333				1,375					1,527				1,650			
Grand Totals		8,760				8,608					9,269				10,861			
Average		122 seconds				120 seconds					129 seconds				151 seconds			

During the first six weeks each group of three pigs received alternately the saline extracts of "splenin A" and of "splenin B" from pooled normal serum. During the last six weeks they received extracts of "splenin A" and of "splenin B" from pooled rheumatoid serum. The results are recorded in Table III and Table IV.

TABLE V.—Analysis of Variance (Experiment 2)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Variation due to characteristic bleeding-times of each guinea-pig	1,403	5	281	0.30	N.S.
Variation due to characteristic day effects on the bleeding-time	7,805	10	781	0.85	N.S.
Variation among each set of four observations taken in sequence	401,712	432	936	1.01	N.S.
Effects of Treatments:					
Variation among the four groups of guinea-pigs before treatment	3,267	3	1,089	1.18	N.S.
Decrease in bleeding-time due to:					
Normal serum extract A	13,806	1	13,806	14.96	0.001 > P
Normal serum extract B	1,008	1	1,008	1.09	N.S.
Rheumatoid serum extract A	160	1	160	0.17	N.S.
Increase in bleeding-time due to rheumatoid serum extract B	17,600	1	17,600	19.07	0.001 > P
Residue remaining after removal of the above effects regarded as error arising randomly	111,731	121	923	—	
Total variation from all sources	558,492	575			

The data in Tables III and IV gave the variance analysis shown in Table V. From this it can be seen that the six guinea-pigs were more uniform than those used in the first experiment (variance ratio=0.85, $n_1=5$, $n_2=121$, not significant). The twelve occasions on which the experiment was carried out did not give rise to differences in bleeding-times (variance ratio=0.85, $n_1=10$, $n_2=121$, not significant). The four groups of observations before treatment did not vary more than would be expected ($F=1.18$, $n_1=3$, $n_2=121$, not significant). After treatment with serum extracts the groups differed. Table VI shows the differences.

TABLE VI

Treatment	Initial Bleeding-time	Alteration in Bleeding-time	Responding Bleeding-time
Normal serum extract A	122 seconds	Decreased 20	102 seconds
Normal serum extract B	128 "	" 5	123 "
Rheumatoid serum extract A	122 "	" 2	120 "
Rheumatoid serum extract B	129 "	Increased 22	151 "

and from the variance analysis (Table V) it appears that normal serum extract A decreases the bleeding-time (variance ratio=14.96, $n_1=1$, $n_2=121$, $P=0.001$) and rheumatoid serum extract B increases it (variance ratio=19.07, $n_1=1$, $n_2=121$, $P=0.001$). The remaining two treatments were ineffective at the dose given (variance ratio=1.09 and 0.17, respectively, $n_1=1$, $n_2=121$, not significant).

Conclusions

It is concluded that a factor or group of factors present in the "splenin A" extract of normal serum reduces bleeding-time, but is absent from a similar

extract from rheumatoid patients. A factor or group of factors is present in the "splenin B" extract of rheumatoid serum that increases bleeding-time, but is absent from normal serum. Both effects have been found consistently.

The experiments, while confirming the existence of the substances called by Ungar "splenin A" and "splenin B," give no indication of the manner or place of production of these substances. His view that they are produced in the spleen and that their production forms part of the general adaptation syndrome is at present under investigation.

We wish to express our thanks to Dr. G. Ungar for initiating us into his methods of extracting the "splenins"; to Mr. A. L. Bacharach and Mr. B. Basil, both of Glaxo Laboratories Ltd., for their help in planning this experiment and undertaking the statistical analysis, respectively; and to Dr. W. J. Martin, of the Statistical Research Unit, London School of Hygiene and Tropical Medicine, for his helpful criticism.

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THE STERILIZATION OF WOOLLEN FABRICS

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Air-borne infection is frequent in the case both of wounds and of infections of the respiratory tract. The infectious organisms are carried on dust or on droplets and droplet nuclei arising from the mouth and nose. Of the two, dust is probably responsible for greater degrees of air contamination (Hare and MacKenzie, 1946; Duguid and Wallace, 1948). Such infected dust arises for the most part from the movement of fabrics—for example, blankets, clothes (van den Ende, Lush, and Edward, 1940; Green, Challinor, and Duguid, 1945; Duguid and Wallace, 1948), bandages (Bourdillon and Colebrook, 1946), and handkerchiefs (Dumbell, Lovelock, and Lowbury, 1948), and to a somewhat less extent from floor-sweeping (Green, Challinor, and Duguid, 1945), although much of this floor dust has come in the first place from fabrics.

Dust-borne bacteria are relatively resistant to the bactericidal action of ultra-violet light and chemical aerosols (Twort, Baker, Finn, and Powell, 1940; Dumbell, Lovelock, and Lowbury, 1948). Correct ventilation can clear the air fairly rapidly (Bourdillon and Colebrook, 1946). To a large extent, however, control has depended upon preventing the release of dust, by oiling in the case of blankets (Thomas and van den Ende, 1941) and floors (van den Ende, Lush, and Edward, 1940), by adequate gowning in the case of clothes (Duguid and Wallace, 1948), and also, of course, by washing.