TABLE III.—Haemoglobin Frequencies in East Anglia Blood Donors

	Civilians		H.M. Forces		Totals			
	M	F	M	F	M	F	Civ.	H.M.F.
No. examined	11,089	6,830	7,241	381	18,330	7,211	17,919	7,622
No. with anti-haem }	1,113 10%	523 7.7%	421 5.8%	21 5.5%	1,534 8.4%	544 7.5%	1,636 9.1%	442 5.8%

TABLE IV.—Age Distribution

Years:	20	20-29	30-39	40-49	50+
No. examined	2,179	9,588	5,231	4,699	3,844
No. with anti-haem }	114 5.2%	570 5.9%	498 9.5%	472 10.1%	424 11.0%

Anglian Blood Transfusion Service. It will be seen that 2,078 (8.1%, S.D. 0.16) gave a positive result. There was a general tendency among males and females, civilians and Service personnel, for the frequency of haemolysins to increase with advancing years, and in all age groups the proportion of males showing haemolysins was higher than that of females; but in this series the difference was not significant at the 3 S.D. level. Out of 18,330 males, 1,534 were positive (8.4%, S.D. 0.205) against 544 females out of 7,211 (7.5%, S.D. 0.31).

Among 17,919 civilians, aged 18-65, 1,636 (9.1%, S.D. 0.215) were positive, but only 442 out of 7,622 members of H.M. Forces (5.8%, S.D. 0.275), and this is decidedly significant at the 3 S.D. level. We are unable to find evidence that the effect of the routine inoculations received by members of the armed Services has been to raise the frequency of anti-A haemolysins among group O Services donors to an appreciable extent as compared with the frequency in the civilian population who attend at civilian or industrial donor sessions; and since the detection of haemolysins *in vitro* is so easy, we do not support the suggestion of Crawford and her associates that all such donors should be refused without investigation. We think that any effect of inoculations in raising the frequency of anti-A haemolysins in Services donors is more than offset by the predominance of younger persons in these populations, and, as we have seen, the frequency is low at the younger ages. We think that the problem raised by these donors, whether occurring in H.M. Services establishments or elsewhere, is a small one, and can be adequately met by the routine screening of all group O donors for anti-A haemolysin, and the labelling of the donations in which it is observed, so that these donations will be used only for group O patients, after satisfactory cross-matching.

Summary

A test suitable as a routine screening test for the detection of anti-A haemolysin in group O donors is described, using the serum sample collected for the routine grouping and Wassermann testing of the donation, and using horse serum as a source of complement.

In a series of 25,541 consecutive group O donations there was a tendency for the frequency to increase with advancing years. The effect of vaccines and similar injections to members of the armed Services was not sufficient for the frequency among them to increase to a significant figure. In our series of nearly 18,000 civilians and over 7,600 members of H.M. Forces it was actually lower in the Services than among civilians.

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We think, however, that this merely reflects the preponderance of young donors at the Services sessions.

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ON THE IMMUNOLOGICAL REACTIVITY OF TUBERCULOUS PATIENTS

PRELIMINARY REPORT

BY

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Greenwood, Smellie, Barr, and Cunliffe (1958) published the results of a study concerning the immunization of sarcoidosis patients against tetanus. Their object had been to ascertain how far these patients reacted "normally" to the administration of an antigen. Their choice fell on the tetanus antigen (fluid tetanus toxoid) because on practical grounds (sporadic occurrence of tetanus) these patients might be assumed not to have come into contact with this antigen before, just like non-immunized healthy controls; in other words, they might be expected not to have acquired a potential immunity for this antigen.

Their sarcoidosis patients and non-immunized controls received two intramuscular injections of fluid tetanus toxoid at an interval of six weeks. In both groups the reaction to this immunization was ascertained by determining the antitoxin in the blood serum a fortnight after the second antigen injection.

During this primary immunization the sarcoidosis patients formed much less antitoxin than the normal controls. Sarcoidosis patients who had been immunized against tetanus some years before, and with whom the tetanus toxoid administered during the experiment therefore acted as a "booster injection," reacted to it like healthy persons.

We have been engaged for a considerable time in an investigation of the reactions of tuberculous patients to immunization. The results recorded by Greenwood

TABLE I.—*Sex and Age of Patients and Controls*

No.	Sex	Age	No.	Sex	Age
<i>Tuberculous Patients</i>					
1	Male	34 years	9	Male	50 years
2	Female	24 "	10	"	37 "
3	"	47 "	11	"	22 "
4	Male	42 "	12	"	45 "
5	"	47 "	13	"	50 "
6	"	35 "	14	"	15 "
7	"	50 "	15	"	50 "
8	"	42 "	16	Female	33 "
<i>Controls</i>					
1	Male	27 years	7	Male	35 years
2	"	26 "	8	Female	20 "
3	"	35 "	9	"	24 "
4	Female	22 "	10	"	22 "
5	"	38 "	11	"	25 "
6	Male	23 "			

et al. made us decide to publish a preliminary report on our own results. A detailed study will be published later on. On the same grounds as the English investigators mentioned above, we chose the tetanus antigen for our experiment.

Clinical Material

The experiment comprised a total number of 16 tuberculous patients and 11 healthy controls. Their age and sex are shown in Table I. They were given two intramuscular injections of tetanus phosphate toxoid ("tetanus P.T."). This vaccine consists of purified tetanus toxoid adsorbed on aluminium phosphate (10 Lf tetanus toxoid and 3 mg. AlPO_4 per ml.). The dose injected was always 0.5 ml. The interval between the two injections was three to five weeks for the tuberculous patients and three to four weeks for the controls. At set times—namely, before the first P.T. injection, at the moment of the second antigen injection and one, two, three, four, five, six, and seven weeks afterwards—the antitoxin titres in the blood serum of the persons treated were determined.

The sera whose titres were expected to be ± 0.001 unit/ml. were titrated on mice at the $L+10,000$ level in the following series: 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256 unit/ml.

After being injected the mice were observed for four days. The intermediate values were interpolated logarithmically. If the titres were expected to be higher than those mentioned above, the sera were titrated at $L+1,000$ or $L+100$, the logarithmic series used being in principle the same—that is, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 and 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.40, 12.8 units/ml.

Results

The results of this experiment are given in Tables II and III.

Analysis of Results*

One is struck at first sight by the great difference in titre values between the tuberculous patients and the controls. If one takes, for instance, the value found one week after the second injection of vaccine it will be seen that 13 of the 16 tuberculous patients do not show any reaction. Not a single markedly increased titre value was found. Only for 2 out of 16 patients were titre values found of more than 0.01 unit/ml.; in one case no figure is given. The control group presents quite a different picture. Here we see that one week after the second injection of vaccine 6 of the 11 members of the group reached a titre value of more than 0.01 unit/ml.

Wilcoxon's significance test shows that the chance that this may just be an accidental difference is so slight as to be negligible ($P < 0.0001$). In view of the data at our disposal we may therefore say that it is

*The statistical analysis was carried out by De Nederlandse Stichting voor Statistiek at The Hague. A special word of thanks is due to Mr. J. J. M. van Tulder, who undertook this analysis.

TABLE II.—*Immunization of Tuberculous Patients*

Case No.	Characterization of Process	Titre at First Antigen Injection	Weeks between First and Second Antigen Injection	Titre at Second Antigen Injection	Titre (Units/ml.) after Second Antigen Injection						
					1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks	7 Weeks
1	Recent	< 0.001	4	< 0.001	< 0.001	—	—	0.032	—	—	—
2	Chronic	< 0.001	4	0.008	0.011	—	—	0.023	—	—	—
3	"	< 0.001	4	< 0.001	—	—	—	0.008	—	—	—
4	"	< 0.001	4	< 0.001	0.001	< 0.001	0.002	0.002	< 0.001	0.001	—
5	Recent	0.001	4	< 0.001	< 0.001	0.001	0.004	0.002	0.001	0.006	—
6	Chronic	< 0.001	4	< 0.001	< 0.001	< 0.001	—	0.008	< 0.001	< 0.001	—
7	"	< 0.001	4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	—
8	"	< 0.001	4	0.001	< 0.001	0.003	0.008	0.016	0.045	—	—
9	"	< 0.001	4	< 0.001	< 0.001	0.090	0.362	0.640	0.320	0.320	—
10	"	0.002	4	< 0.001	0.023	0.016	0.090	0.090	0.128	0.032	0.016
11	Recent	< 0.001	4	< 0.001	< 0.001	0.128	0.070	0.080	0.011	—	—
12	Chronic	< 0.001	5	< 0.001	0.001	0.001	0.003	0.003	0.003	0.003	—
13	"	0.001	5	< 0.001	0.003	0.032	0.032	0.032	0.032	0.006	—
14	Recent	< 0.001	5	< 0.001	< 0.001	0.001	0.001	< 0.001	< 0.001	0.001	—
15	"	< 0.001	5	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	—
16	Chronic	< 0.001	3	< 0.001	0.001	0.003	< 0.001	—	< 0.001	—	—

TABLE III.—*Immunization of Healthy Controls*

Case No.	Titre at First Antigen Injection	Weeks between First and Second Antigen Injection	Titre at Second Antigen Injection	Titre (Units/ml.) after Second Antigen Injection						
				1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks	7 Weeks
1	< 0.001	4	< 0.001	< 0.001	—	0.181	—	0.900	0.300	—
2	< 0.001	4	< 0.001	< 0.001	—	—	1.81	—	—	1.60
3	< 0.001	4	< 0.001	< 0.001	0.064	0.320	0.032	0.032	—	—
4	< 0.001	4	< 0.001	3.60	—	—	2.56	—	2.30	2.30
5	< 0.001	4	< 0.001	0.064	0.256	0.600	1.10	0.600	0.230	0.160
6	< 0.001	3	< 0.001	0.008	0.011	0.020	—	0.016	—	—
7	< 0.001	3	< 0.001	0.045	0.045	0.040	—	0.023	—	—
8	< 0.001	3	< 0.003	0.011	0.110	0.080	—	0.160	—	—
9	< 0.001	3	< 0.001	0.002	0.045	0.040	—	0.011	—	—
10	< 0.001	3	< 0.001	0.032	0.160	0.110	—	0.040	—	—
11	< 0.001	3	0.003	0.128	0.040	0.040	—	0.110	—	—

practically certain that these antigen injections have less immunizing effect on tuberculous patients than on healthy controls.*

Our data are not extensive enough to allow of further analyses, especially with respect to a possible difference between the effect of immunization on acute and on chronic patients. Any differences there may be cannot be shown to be statistically significant. There is some indication that the tuberculous patients consist of two groups—that is, those who still do not show any increased titre values after from three to six weeks and those who reach fairly high titre values two to three weeks after the second injection of antigen. Or, to put it differently, there are tuberculous patients who did not react at all throughout the experiment and there are patients who did react albeit somewhat tardily, while at the same time the level of the titre values for the latter may be lower than with healthy persons.

However, these are but vague indications, and only a more extensive investigation can show what they are worth.

Discussion

Our findings with regard to the immunization of tuberculous patients appear to be essentially the same as the results obtained with sarcoidosis patients by the English investigators mentioned above.

Consequently the question now arises whether a chronic disease—that is, sarcoidosis or tuberculosis—causes a lesion or an exhaustion of the reticulo-endothelial system (R.E.S.) (which, we believe, plays an all-important part in the formation of antibodies). Greenwood *et al.* assume that the sarcoidosis infiltrates the R.E.S., as a result of which there can be no adequate reaction to the antigen stimulus (any more).

On the other hand, there is the possibility of there being a primary disorder of the R.E.S. which has caused the inability to resist these diseases.

Our limited material comprises both very recent and chronic infections.

The reaction pattern we found showed no difference in the antibody production of the two groups of patients. So the analysis of the antitoxin formation after the administration of the two tetanus P.T. injections did not enable us to find any correlation with the clinical picture.

Summary

A number of tuberculous patients were immunized against tetanus with two injections of tetanus phosphate toxoid. Compared with a control group consisting of normal healthy persons these tuberculous patients showed in general a diminished antibody production.

REFERENCE

Greenwood, R., Smellie, H., Barr, Mollie, and Cunliffe, A. C. (1958). *Brit. med. J.*, 1, 1388.

APPENDIX

All sera from patients were titrated according to a logarithmic scale. For practical reasons this logarithmic scale was divided into three subscales:

Scale 1: 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256 unit/ml. of serum.

*It should be noted that actually this conclusion may only be drawn if the 16 tuberculous patients and the 11 controls have been taken at random. As it is usually very difficult to fulfil this condition in investigations like the present, the general validity of this interpretation must as yet be accepted with some reserve.

Scale 2: 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 unit/ml. of serum.

Scale 3: 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.40, 12.8, unit/ml. of serum.

So each scale gives nine titre values (scale 3 only eight). From all serum-toxin-saline mixtures (4 ml.) 0.4 ml. was injected subcutaneously into a mouse.

Every series of titrations of an unknown serum was compared with a control titration with standard serum. For this reason our own standard serum (titrated against the International Standard Serum from the Statens Serum Institute, Copenhagen) was used.

For titrations according to scale 1 the standard serum was diluted to 0.001 unit/ml.; for titrations according to scale 2 the dilution was to 0.01 unit/ml., and for scale 3 to 0.1 unit/ml.

To 1 ml. of this standard serum dilution was added the corresponding quantity of test toxin and the total volume of the mixture brought to 4 ml. with saline. Of this mixture, 0.4 ml. was injected subcutaneously into a mouse. This last-mentioned quantity of the mixture therefore contained 0.0001 viz. 0.001, 0.01 unit, and so much toxin that the mice normally died four days after the injection. These toxin quantities therefore were L+/10,000, viz. L+/1,000. L+/100.

To get a good insight into the course of the serum titrations, and especially to obtain a good check of the "control mixtures," all titrations with the diluted standard serum were performed with three different quantities of diluted standard serum (D.S.S.):

0.9 ml.	D.S.S.+1	L+/10,000	(1,000, 100) test toxin
1.0 ml.	"	+1 L+/10,000	(1,000, 100) test toxin
1.1 ml.	"	+1 L+/10,000	(1,000, 100) test toxin

All mixtures were made up to 4 ml. with saline, and 0.4 ml. was injected into mice.

The injected mice were observed daily for four days.

COMPARATIVE TRIALS OF NEW ORAL PROGESTOGENIC COMPOUNDS IN TREATMENT OF PREMENSTRUAL SYNDROME

BY

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Progesterone usually provides complete symptomatic relief in cases of premenstrual syndrome (Greene and Dalton, 1953). Against this successful treatment must be set the high cost of progesterone and the inconvenience and discomfort of frequent deep intramuscular injections. Several progestogenic compounds have recently been developed which, being oral preparations, could conveniently replace progesterone if they were as completely successful in their action. Two of these new progestogenic compounds were tested in a comparative trial of 58 patients suffering from premenstrual syndrome who had all obtained complete symptomatic relief with progesterone.

The oral preparations used were ethisterone (anhydro-hydroxyprogesterone or ethinyltestosterone) and the two new oral preparations norethisterone (anhydrohydroxy-19-norprogesterone or ethinylnorprogesterone) and