ENHANCEMENT OF MANTOUX REACTION COINCIDENT WITH TREATMENT WITH CORTISONE AND PREDNISOLONE

BY

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Patients with advanced pulmonary or miliary tuberculosis may show a low or absent hypersensitive reaction to intradermal injections of tuberculin (Happ and Casparis, 1922; Hart, 1932). Such patients often show excessive pigmentation of the skin with a distribution reminiscent of that seen in suprarenal insufficiency, and there is some support for this clinical observation in the work of Clarke *et al.* (1954), who showed a correlation between the severity of the disease in patients suffering from pulmonary tuberculosis and the urinary 17-ketosteroid excretion. In their series of 109 patients, those with the more frankly exudative disease had the lowest excretion of 17-ketosteroids. Of the patients classified as suffering from far-advanced disease, 86% had a low 17-ketosteroid excretion, and in 37% the excretion was less than 50% of the normal. If other factors can be excluded the question arises whether there is any relation between the loss of tuberculin hypersensitivity and insufficiency of suprarenal function.

The investigation of a patient with Addison's disease afforded an opportunity of testing this rather speculative idea. The patient was a 40-year-old woman presenting in pregnancy with hypoglycaemic manifestations of Addison's disease probably secondary to hypopituitarism, and has been reported in detail elsewhere (McFarlane and Truelove, 1957). Two weeks post partum the Mantoux reaction was negative at a dilution of 1/100, and treatment with cortisone was begun as there were definite signs of impending Addisonian crisis. Thirty-seven days later, when the patient was on a maintenance dose of 50 mg. of cortisone daily, the Mantoux reaction had become positive at 1/1,000. This result prompted the observation of the tuberculin sensitivity in patients suffering from various conditions before and during treatment with corticosteroids.

Material and Methods

The results from 24 patients are analysed. They were mostly acute admissions to two general medical wards, and were mostly elderly, although younger ages were represented. Patients who were likely to need steroid treatment were Mantoux-tested routinely, those showing positive reactions to the dilutions employed being excluded. The series includes all patients fulfilling these criteria who were admitted to two wards in a 15-months period, together with three other patients kindly referred to me from elsewhere.

Mantoux tests were performed in the usual way by injecting 0.1 ml. of old tuberculin (Evans) intradermally into the volar surface of the forearm, the reactions being confirmed by independent observers. Time did not always allow the use of serial dilutions, but 1/1,000 was the weakest used. Reactions were classified as positive only if there was definite induration at 48 hours.

During the times concerned the patients received diet, fluid intake, and drugs appropriate to their condition, and there was no constant factor likely to have influenced the results.

Results

Mantoux-testing was done as opportunity offered, and it must be emphasized that the times at which conversion was observed were not necessarily the earliest at which it actually occurred. Late development of reaction at the site of earlier, negative, tests was observed after steroid treatment was started in two cases (Nos. 16 and 17).

The results of 20 cases in which "conversion" occurred are summarized in the Table. It will be noted that three of them changed from negative at 1/100 to positive 1/1,000. The tests recorded in the table as "before treatment" were the lowest dilutions tried, and those "after treatment" were the highest dilutions tried.

On the whole the patients improved in general condition during the period under review, but this was pronounced in only six (Cases 1, 2, 4, 5, 16, and 17). In six (Cases 8, 10, 11, 12, 15, and 19) there was no change; three (Cases 11, 12, and 16) were severely ill and subsequently died; three (Cases 8, 10, and 15) were not very ill. The skin was of normal texture in 10 cases, but in 10 it was dry or showing a senile atrophy. None of the cases converting from 1/100 negative to 1/1,000 positive were among these 10, and only one (Case 5) was among those showing marked clinical improvement. In five cases it was possible to repeat the Mantoux test after steroid treatment was stopped. Three of these became negative to the dilutions which previously gave a positive reaction (Case 2 in three weeks, Case 4 in six days, and Case 11 in eight days), and Case 7 reverted from positive to 1/1,000 to negative at 1/100 in 10 days. Case 13 was still positive

Summary of 20 Cases in which "Conversion" occurred

Case No.	Sex and Age	Diagnosis	Mantoux Before Treatment	Treatment Dosage/Day	Mantoux During Treatment	Time Interval Between Tests
1	M 68	"Rheumatoid disease." Diabetes mellitus. Adrenal failure	Neg. 1/1,000	C. 50 mg.	Pos. 1/1,000	12 months
2	M 73	Bronchopneumonia. Chronic bronchitis. Old chronic pulmonary tuberculosis	" 1/100	C. 50 "	" 1/100	17 days
3	M 51	Bronchopneumonia. Chronic bronchitis	" 1/1,000	P. 15 "	" 1/1,000	8 "
4	F 56	Rheumatoid arthritis	" 1/1,000	C. 25 "	" 1/1,000	7 "
5	F 40	Hypopituitary adrenal failure	" 1/100	C. 50 "	" 1/1,000	37 "
6	F 66	Ulcerative colitis	" 1/1,000	P. 20 "	" 1/1,000	15 "
7	F 75	Rheumatoid arthritis	" 1/100	P. 20 "	" 1/1,000	7 "
8	F 69	Chronic fibroid pulmonary tuberculosis	" 1/1,000	P. 10 "	" 1/1,000	9 "
9	F 73	Giant-cell arteritis	" 1/100	P. 20 "	" 1/1,000	6 "
10	M 68	Tracheobronchitis. Ischaemic heart disease	" 1/1,000	P. 15 "	" 1/1,000	15 "
11	M 55	Chronic bronchitis. Cor pulmonale	" 1/1,000	P. 20 "	" 1/1,000	15 "
12	M 72	Chronic pulmonary tuberculosis. Pulmonary artery thrombosis	" 1/1,000	C. 75 "	" 1/1,000	7 "
13	M 67	Chronic bronchitis. Bronchopneumonia	" 1/100	P. 20 "	" 1/100	14 "
14	F 77	Rheumatoid arthritis	" 1/100	P. 20 "	" 1/100	8 "
15	M 72	Giant-cell arteritis	" 1/100	P. 15 "	" 1/100	2 "
16	M 59	Chronic bronchitis. Psoriasis. Ischaemic heart disease.	" 1/100	C. 50 "	" 1/100	2 "
17	M 80	Rheumatoid arthritis	" 1/100	P. 20 "	" 1/100	2 "
18	M 24	Giant-cell arteritis	" 1/1,000	P. 20 "	" 1/1,000	13 "
19	M 61	Reiter's syndrome	" 1/100	C. 50 "	" 1/100	4 "
20	F 60	Cor pulmonale. Bronchopneumonia	" 1/1,000	P. 15 "	" 1/1,000	47 "
		Bronchiectasis. Chronic fibroid tuberculosis				

C. = Cortisone. P. = Prednisolone.

at the previous dilution of 1/100 18 days after treatment was stopped.

Four of the cases showed definite signs of suprarenal cortical insufficiency (Nos. 1, 4, 5, and 16). Cases 4 and 5 had 24-hour urinary 17-ketosteroid excretion of 1 and 2 mg. respectively (both women : Case 1 had clinical evidence, together with lack of eosinophil responses to corticotrophin 100 units daily ; and Case 16 had electrolyte changes and pigmentation).

With the exception of the Addisonian Cases 4 and 5 there were only four patients under 60 years of age in this series ; one (Case 11) was severely ill, and another (Case 3) was moderately ill but made a fair recovery. Nevertheless, the return of tuberculin hypersensitivity in association with recovery of suprarenal function may occur in a young person undergoing a severe illness, as the following case history shows.

A man aged 35, previously well and with no relevant past history, was admitted as an emergency case of pneumonia following an upper respiratory infection. Five days after admission there were frank signs of a pleural effusion and he subsequently developed an empyema. Mantoux 1/1,000 was negative on admission, and 1/100 was negative 16 days after admission. At this stage he was still severely ill with swinging fever. Urinary 17-ketosteroids were 7.6 mg./24 hours on admission, but by the time of the second negative Mantoux test they had risen to 19 mg./24 hours. Clinical signs of suprarenal insufficiency in the form of hyperpigmentation, including the palmar creases, and fall in blood pressure became evident 10 days after admission, and were still present at the time of the second Mantoux test, although the 17-ketosteroid excretion had risen. The clinical picture, however, was suggestive enough for the decision to be made to give cortisone if there was any further deterioration. Clinical recovery dated from this time. Mantoux 1/100 was still negative 44 days after admission, when the 17-ketosteroid excretion was 16.3 mg./24 hours ; but the Mantoux reaction became positive at 1/100 eight days later, and positive at 1/1,000 when tested after a further one month's convalescence.

In addition to the cases described above, there were four which showed no tuberculin hypersensitivity change at any time.

Case 21.—A woman of 74 with carcinoma of the stomach and rheumatoid arthritis proved negative to 1/100, and was treated with prednisolone 60 mg. daily. Repeated testing showed no reaction whatever.

Case 22.—A woman of 65 with rheumatoid arthritis and fixed joints treated with prednisolone 20 mg. daily and negative to 1/1,000. Further testing with 1/1,000 showed no reaction after seven days.

Case 23.—A man of 72 with chronic bronchitis and emphysema, negative to 1/100, was treated with prednisolone 20 mg. daily. Repeated tests at all dilutions remained negative.

Case 24.—A man of 40 with long-standing bronchial asthma, negative to 1/100 and treated with prednisolone 20 mg. daily. Further testing after eight days showed no reaction.

Case 24 was interesting in that, although this patient showed no reaction to intradermal tuberculin, scratch-testing with standard allergens (Bencard), which were all negative on admission, showed a definite sensitivity to certain of the pollen preparations after seven days' treatment, suggesting that the treatment may have some influence on this form of hypersensitivity also.

Discussion

The results suggest that the Mantoux reaction may be suppressed in certain severe conditions and also that the reaction may return with recovery. This agrees with previous work (Pilcher, 1930 ; Schwartz and Heise 1931). Most patients in the series were elderly, but the phenomenon was observed in some younger patients and has also been described by Taylor, Smith, and Vollum (1953) in younger people with tuberculous meningitis.

The wide variety of conditions investigated and the variations in their clinical states demand a very large control series if the effects of steroid therapy on the Mantoux reaction are to be separated without question from changes merely associated with clinical recovery. Such a control

series was not possible under the conditions of the present investigation, but the results do not suggest a necessary correlation between the return of a suppressed reaction and clinical improvement. Thus six of the cases showed no clinical improvement during the period under review, and the rapidity of the conversion observed in some cases is against any such correlation.

Wasting and dehydration are commonly accepted as simple causes of lack of skin reactivity which may result in a negative tuberculin reaction. There is no evidence that these factors influenced the present series, as there were mostly no changes in the condition of the skin during the relevant times. Finally, the mere performance of tuberculin-testing may enhance a previous reaction (O'Grady, 1957), but there is no evidence that this was a significant factor in this series, and, as described, four cases became negative again in spite of repeated testing. In one case (No. 17) a reaction, previously negative, became rapidly positive following administration of prednisolone, without further injection of tuberculin.

It is generally accepted that cortisone may, in therapeutic dosage, suppress the Mantoux reaction (Long and Favour, 1950 ; LeMaistre *et al.*, 1951 ; Long and Spensley, 1954 ; Houghton and Davies, 1954) ; and the anti-inflammatory action of cortisone is established. However, Coste *et al.* (1951) treated 15 patients with arthritic or rheumatic conditions with enhancement of the tuberculin reaction in 10 ; and Sulzberger *et al.* (1951) reported that of three patients with negative reactions two became positive after treatment with corticotrophin. The effect of treatment with small doses of steroid hormones on the Mantoux reaction has not been previously described.

In certain circumstances the addition of small quantities of cortisone to the tuberculin extract may result in the enhancement of the reaction or at least result in a diminution of the usual inhibitory effect. Citron and Scadding (1957) have shown a direct relationship between the degree of inhibition of the response by local cortisone and the degree of hypersensitivity present. They also confirm earlier work (Pyke and Scadding, 1952), in which it was shown that a negative tuberculin response in sarcoidosis may become positive with cortisone treatment or if cortisone is added to the tuberculin reagent. They postulate that these effects may be due to retention of the tuberculin at the injection site for a longer period than usual.

The treatment of pulmonary tuberculosis with steroid hormones in addition to antibiotics is rapidly becoming established (Houghton, 1954 ; Rose and Jauffret, 1956 ; Favez and Aguet, 1956), and the advantages have been assumed to be related to suppression of the hypersensitive reaction. However, Even *et al.* (1955), while supporting this method of treatment, recommend the use of more "physiological" doses than those reported as causing spread of infection. The reactivation of chronic tuberculous lesions in men over 65 has recently received attention (Wilkins, 1956), and this, together with the results of Clarke *et al.* (1954), who found low 17-ketosteroid excretion especially in elderly patients, suggests that in certain cases the advantages gained by steroid treatment in small dosage may be due to the correction of the effect of suprarenal insufficiency rather than to the suppression of a hypersensitive response. If this is so it may be expected that other severe infections apart from tuberculosis would respond, especially in the elderly, to the addition of steroid hormones in small dosage to standard antibiotic treatment. Such treatment was used in Case 2, in which the primary disorder was a severe pyogenic bronchopneumonia, and the response was clinically dramatic. This patient also had two relapses of his chest infection in the following year. On each occasion the relapse closely followed the reduction or stoppage of cortisone, and both times recovery was rapid when the drug was recommenced.

The small number of cases in this series and the lack of adequate controls make the results inconclusive, but the implications seem to justify publication as a preliminary report.

Summary

The cases are presented of 24 patients with various diseases who had negative tuberculin reactions. Whilst treated with cortisone or prednisolone in moderate or low dosage the tuberculin reaction became positive at the same or higher dilutions in 20 of the cases.

The patients were mostly elderly, or showed frank signs of suprarenal cortical suppression.

The suggestion is made that steroid treatment, although able to suppress a positive tuberculin reaction when given in high dosage, may, given in lower dosage, actually restore a reaction which has been suppressed by age, infection, or suprarenal deficiency.

The implications of these findings and their possible application to the treatment of severe infections, especially in the elderly, are discussed.

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Speaking at a conference on mental deficiency at Manchester on November 7 and 8, Mr. RICHARD THOMPSON, Parliamentary Secretary to the Ministry of Health, said that the theme of the Royal Commission's proposals was based on the success of non-residential training and a growing tolerance of the public towards the mentally handicapped. To obtain results a closer co-ordination between health, welfare, and education services was essential, because the needs of defectives were chronic but not constant. If community care was to work public opinion must accept it, which meant abandoning some of the controls and restraints of present procedures. "We should develop a new outlook towards mental defectives not as hopeless outcasts, as has so often been the case hitherto, but more as we consider, for instance, blind people." Great progress had been made since the start of the Health Service in the organization for training mental defectives at occupation centres. In 1948 100 centres existed; to-day over 300. In 1951 just over 4,000 boys and girls under 16 were receiving training; to-day well over 9,000. Because most resources had been applied to mentally handicapped children, the more complicated problems of adult defectives had not yet been properly studied. With full employment and economic stringency it was difficult to provide staff and buildings, but this need not prevent a start being made. The conference was arranged by the National Association for Mental Health.

THE BINDING OF HAEMOGLOBIN BY PLASMA PROTEINS (HAPTOGLOBINS)

ITS BEARING ON THE "RENAL THRESHOLD" FOR HAEMOGLOBIN AND THE AETIOLOGY OF HAEMOGLOBINURIA

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Jayle and his colleagues (Jayle, 1939, *et seq.*, see Jayle and Boussier, 1955) have carried out an extensive series of investigations on serum proteins that have the striking property of combining with haemoglobin. These proteins have been termed haptoglobins, from the Greek root $\alpha\pi\tau\epsilon\iota\nu$, meaning "to hold fast." The haptoglobin migrates with the α_2 fraction on electrophoresis in the Tiselius apparatus and on filter paper, but when haemoglobin is added the haptohaemoglobin complex has a mobility corresponding to that of β_1 -globulin (Jayle, Boussier, and Badin, 1952). This observation has been confirmed by Wieme (1953), Tuttle (1955), and Reich (1956). Tuttle failed to find the haptoglobin in umbilical cord blood, although it was present in the blood of older children.

In an interesting series of experiments, Smithies (1955a, 1955b) observed that when mixtures of haemoglobin and serum are submitted to electrophoresis in starch gels the resulting patterns differ from subject to subject in a manner described fully below. Since the haptoglobins appear to be the only serum proteins that combine with haemoglobin, there is little doubt that Smithies was describing individual differences in haptoglobins. Smithies reported that the haptoglobins form a complex with about 125 mg. of haemoglobin per 100 ml. of plasma, any excess of haemoglobin above this figure remaining uncombined. Smithies, like Tuttle, was unable to find any haptoglobins in umbilical cord blood.

We have been able to confirm and extend the findings of Smithies and have obtained evidence that the binding of haemoglobin by plasma proteins has a bearing on the circumstances under which methaemalbumin is formed, on the problem of the so-called "renal threshold" for haemoglobin, and on the aetiology of haemoglobinuria.

Material and Methods of Investigation

Serum specimens from 12 normal subjects and 7 patients with haemoglobinuria have been studied. About 5 ml. of blood was allowed to run down the side of a centrifuge tube. The clot was allowed to form and retract for six hours at room temperature, and serum was removed after centrifugation. The serum proteins were submitted to electrophoresis in starch gels in borate buffer, pH 8.48, using the technique of Smithies (1955b). After electrophoresis the gels were sliced horizontally into two parts, one of which was stained with amidoschwartz B (Gurr) while the other was stained for haem proteins by benzidine (Franklin and Quastel, 1949) or