EFFECT OF CORTISONE UPON SKIN SENSITIVITY TO TUBERCULIN IN SARCOIDOSIS

BY

D. A. PYKE, M.B., M.R.C.P.

AND

J. G. SCADDING, M.D., F.R.C.P.

(From the Department of Medicine, Postgraduate Medical
School of London, W.12, and the Institute of
Diseases of the Chest, Brompton)

The observations reported in this paper were started when, in a patient suffering from sarcoidosis and receiving cortisone, a change in the skin sensitivity to tuberculin was noticed. This patient showed a diffuse nodular infiltration of the lungs, histological changes typical of sarcoidosis on biopsy of the liver, and a persistently negative Mantoux reaction, no reaction having been evident at any time after the intradermal injection of 100 T.U.* of old tuberculin (O.T.) on several occasions during the preceding six months. Cortisone was administered intramuscularly in a dose of 100 mg. daily. Forty-eight hours before this treatment was started 100 T.U. of O.T. had been injected intradermally, and had produced no reaction when read at the usual interval of 48 hours. On the fourth day of cortisone administration a small flare was noted at the site of this tuberculin injection. On the fifth day this had developed into an indurated area 25 by 17 mm., which gradually faded within the next two days. Also on the fifth day, another intradermal injection of 100 T.U. of O.T. was made; this produced an indurated area 20 by 25 mm. on the sixth day, which was still present but fading on the seventh day, and did not disappear completely until the eleventh day. On the sixth day 10 T.U. of O.T. was injected. After 24 hours there was a small flare, and at 48 hours an area of induration 10 by 8 mm., which gradually faded over the next two days. On the eighteenth day 100 T.U. of O.T. was injected, but produced no reaction. These reactions resembled normal positive tuberculin reactions, but appeared rather earlier and faded rather more quickly. This experience led us to investigate the effect of cortisone on the skin sensitivity to tuberculin in patients with sarcoidosis.

Clinical Data

We have made observations on 14 patients who showed a clinical picture compatible with sarcoidosis—13 with lung changes and the remaining one with gross hilar lymph-node enlargement; in all of them histological confirmation had been obtained, sarcoid-type tubercles in an active phase having been found in biopsy material. This was obtained from the liver in six cases, from lymph nodes in four, from skin in three, and from a nodule in the breast in one. Although a negative tuberculin reaction was not included in the criteria for diagnosis, it was in fact found in most of the cases investigated. Eleven of the 14 showed no reaction to at least 100 (usually 1,000) T.U. of O.T. or to 250 T.U. of purified protein derivative (P.P.D.); two reacted to 10 T.U. and one to 100 T.U. of O.T.

*Throughout this paper quantities of tuberculin will be stated in terms of tuberculin units (T.U.). 1 T.U. $\equiv$ 0.00002 mg. P.P.D. $\equiv$ 0.1 ml. of 1:10,000 O.T.

Effect of Cortisone Administered Intramuscularly

Cortisone was administered intramuscularly to 10 patients who were Mantoux-negative to at least 100 T.U. The cortisone was given in a single daily intramuscular injection of 100 mg. for 20 days. A dose of tuberculin of the type used for the preliminary Mantoux tests, and equal to or less than that used in these tests, was injected intradermally at intervals during the course of this treatment. The size of any resulting area of induration or erythema was measured at 24 and 48 hours. Some change in skin reactivity to tuberculin was observed at some stage in 8 of the 10 patients.

In six of these (including the patient already described) the reaction was similar in appearance to a normal positive Mantoux reaction. The time at which the change in skin reactivity was first observed varied from case to case. The earliest tuberculin injections to which reaction was detected were made on the third, fifth, fifth, sixth, tenth, and seventeenth days respectively. In one patient skin sensitivity was still present on the nineteenth day, in three it had disappeared by the eighteenth day, and in two no further observations were made. The reactions were accelerated as compared with the normal positive tuberculin reaction. They all reached a maximum by 24 hours, except one which was at its greatest 48 hours after the injection. Although the reactions faded quickly, and had usually disappeared in 72 hours, all these patients had at least one reaction which was still showing an area of induration at 48 hours. Usually the later tests became less intense and faded more quickly.

In two patients the observed skin reaction to tuberculin consisted only of the appearance of erythema, without induration. This constituted a definite change, since there had been no reaction of any sort before cortisone administration. The erythema reached its maximum of 20–30 mm. in diameter by 24 hours after the tuberculin injection, and faded rapidly. In both cases the greatest reaction was observed to an injection made soon after the beginning of cortisone administration. It could not be obtained again on the tenth day or later in one case, and on the thirteenth day or later in the other.

In only two patients was no change in skin reactivity to tuberculin observed at any time during cortisone administration.

Two of the patients who had shown a response early in a course of cortisone administered intramuscularly, and in whom the induced skin reactivity to tuberculin had disappeared later in the course, received courses of cortisone 100 mg. daily by mouth after an interval. In one no reaction to intradermal tuberculin was observed at any time during oral administration of cortisone, started eight weeks after the last intramuscular injection. The other had shown a response to 100 T.U. of O.T. early in the course of intramuscular cortisone, although she normally failed to react to 1,000 T.U. During the oral administration of cortisone, started six weeks after the last intramuscular injection, she showed a wheal 20 mm. in diameter 24 hours after the injection of 1,000 T.U. of O.T. on the fifth and sixth days, a much smaller reaction on the twelfth day, and none on the fifteenth; 100 T.U. produced only erythema 24 hours after injection on the fifth day, but there was no response to this dose of tuberculin later.

Effect of Local Cortisone

In 12 of the 14 cases the effect of cortisone mixed with the tuberculin used for intradermal injection was studied, whenever possible before intramuscular cortisone had been given. The technique used was similar to that of Vollmer (1951). At least three injections were made in each case: a dose of tuberculin equal to that which had previously been used for the determination of tuberculin sensitivity; the same dose of tuberculin with 1.25 mg. of cortisone; and 1.25 mg. of cortisone alone (1.25 mg. of cortisone is contained in 0.05 ml. of the standard suspension). The test

solutions were each made up to a total volume of 0.1 ml. for intradermal injection by adding to 0.05 ml. cortisone suspension 0.05 ml. of tuberculin solution of twice the test strength to make the cortisone-tuberculin injection, and 0.05 ml. of saline solution for the cortisone injection. In four cases a further control, consisting of 0.05 ml. of the aqueous vehicle in which cortisone is suspended diluted to 0.1 ml. with normal saline, was used, but produced no reaction. In a few subjects, not in this series and not suffering from sarcoidosis, we have found a skin reaction to the aqueous vehicle, but this was invariably inhibited by cortisone, and therefore cannot be a confusing factor.

Nine of the 12 patients in whom the effect of local cortisone was tested were normally Mantoux-negative. Seven of these showed changes similar to those obtained with systemic cortisone. Areas of induration with surrounding erythema appeared at the site of the mixed tuberculin and cortisone injection, usually reaching a maximum between 24 and 48 hours, and then fading. At the site of the tuberculin injection there was no reaction. At the site of the cortisone injection a small intradermal nodule, presumably due to the particulate nature of the cortisone suspension, was often observed. This was quite different from the induration of a tuberculin reaction, and had no surrounding erythema.

In the three patients who were Mantoux-positive to 100 T.U. or less the effect of the addition of cortisone to the injection was to inhibit the reaction which was evident at the site of injection of tuberculin alone.

Correlation between Results of Systemic and of Local Administration of Cortisone

Eight out of the 11 Mantoux-negative patients were investigated with both systemic and local administration—five showed an altered response after both methods, two showed a response with local administration only, and one with systemic only. In two cortisone was given by intramuscular injection only, and one of these showed a definite response and one erythema. One patient was tested with local cortisone only, and showed no response. The results are summarized in the Table.

Summary of Effects of Cortisone on Skin Sensitivity to Tuberculin in 14 Patients with Sarcoidosis

Case	Sex	Age (Years)	Original Skin-sensitivity to 100 T.U.	Route of Administration		
				Intramuscular (100 mg. daily)		Local (1-25 mg. with Tuberculin)
				Response	Day of Cortisone Course on which Tuberculin Injection Causing Greatest Reaction was Given	
1	F	43	Negative	+	6	+
2	F	32	"	+	—	+
3	M	34	"	+	6	+
4	F	27	"	+	10	0
5	F	43	"	0	—	+
6	F	31	"	+	7	Not tested
7	M	43	"	±	1	" "
8	F	23	"	±	5	+
9	F	51	"	+	5	+
10	M	33	"	+	17	+
11	F	27	"	Not given	—	0
12	F	34	Positive	" "	—	Inhibited
13	F	28	"	" "	—	"
14	F	27	"	" "	—	"

+ = skin reaction persisting 48 hours in subject normally insensitive to tuberculin.

± = skin reaction persisting 24 hours, but less than 48 hours.

Inhibited = skin reaction to tuberculin inhibited.

Cases Excluded from Analysis

In addition to the 14 patients already described, three patients with proved sarcoidosis have been tested—two with both systemic and local administration of cortisone, and one with local only. For what were considered to be good reasons these cases have been excluded from the analysis presented above. In one case the diagnosis was proved histologically more than three years ago; the histological changes then were those of an inactive phase, and the

residual changes now consist only of probably non-specific residual fibrosis and emphysema in the lungs. The other two presented the complicating factor of having completed a prolonged course of systemic cortisone shortly before the tests were made. All the tests on these three cases were negative.

Discussion

The absence of skin sensitivity to tuberculin which is found in many cases of sarcoidosis is one of the most remarkable features of this syndrome, and has been investigated extensively. It is different in important respects from the lack of sensitivity shown by normal persons who have not been infected with tuberculosis. The reported inability to cause conversion of the negative Mantoux reactions in some cases of sarcoidosis to positive by B.C.G. vaccination (Lemming, 1940; Israel *et al.*, 1950) and the presence of a tuberculin-neutralizing factor ("anticutin") in the sera of some patients with sarcoidosis (Martenstein and Jadassohn, 1923; Wells and Wylie, 1949; Sterling, 1950) are indications of this difference. Neither of these phenomena is constantly found; but each of them has been reported in enough cases to support the view that the negative Mantoux reaction in sarcoidosis may well be more than an indication of the simple absence of tuberculous infection. The phenomenon reported in this paper is likewise inconstant, but it also indicates something peculiar about the negative Mantoux reaction in sarcoidosis. Observations on two patients have failed to show any correlation between the presence of tuberculin-neutralizing factor and the induction of temporary tuberculin sensitivity by cortisone. Specimens of serum from these two patients, in both of whom temporary skin sensitivity to tuberculin was induced by cortisone administered both systemically and locally, were tested by the method of Wells and Wylie. No tuberculin-neutralizing effect was found in sera taken before cortisone treatment or during the phase of tuberculin sensitivity.

In the few healthy Mantoux-negative individuals in whom we have tried the effect of cortisone added to the tuberculin injected for a tuberculin test no modification of the negative test has been observed.

The positive Mantoux reaction found in healthy persons who have had a tuberculous infection, or in sufferers from tuberculosis, is usually inhibited or diminished by cortisone, administered either systemically or locally (Long and Favour, 1950; Le Maistre *et al.*, 1951; Vollmer, 1951; Wallner *et al.*, 1952); we have confirmed this observation and, as recorded above, have found it also to hold true in three patients with the clinical and histological picture of sarcoidosis in whom the Mantoux reaction was positive. The only authors to report enhancement of tuberculin skin sensitivity by cortisone are Coste *et al.* (1951). In some patients under treatment by cortisone or A.C.T.H. for a variety of non-tuberculous diseases but with positive Mantoux reactions they found a slight increase in the size of the reaction, together with acceleration of its appearance. This increase was inconstant and usually of slight degree, and was greatest after prolonged treatment (one month).

In general, cutaneous hypersensitivity of the "tuberculin" type appears to be inhibited, diminished, or unaffected by cortisone; the skin sensitivity to tuberculin induced by systemically or locally administered cortisone in subjects previously insensitive which we have observed in some cases of sarcoidosis appears to be unique. This phenomenon is obviously of the greatest theoretical interest in relation to the aetiology of sarcoidosis, and may prove to be of some practical importance as a diagnostic test; but our present purpose is no more than to record its occurrence.

Summary

The effect of cortisone upon skin sensitivity to tuberculin was investigated in 14 patients with sarcoidosis.

Ten patients who normally failed to react to at least 100 T.U. of tuberculin intradermally received 100 mg.

of cortisone daily by intramuscular injection. In eight of these skin sensitivity to tuberculin was detected at intervals ranging from 6 to 17 days after the first injection of cortisone; in six the reaction produced by tuberculin during the period of sensitivity persisted, with induration, up to 48 hours, and in two it consisted of erythema, evident at 24 hours but fading at 48 hours. In those patients tested repeatedly this sensitivity was transient, even though the cortisone was continued.

In nine patients normally not reacting to at least 100 T.U., intradermal injection of 1.25 mg. cortisone with a dose of tuberculin equal to the greatest to which the patient had previously failed to respond produced a reaction with induration and erythema persisting at least 48 hours in seven, and no response in two.

In three patients normally sensitive to 100 T.U. or less, similar local administration of cortisone with tuberculin inhibited the reaction produced by tuberculin alone.

We are grateful to the house-physicians at Brompton Hospital and Hammersmith Hospital for their co-operation in these observations. Part of the cortisone used was supplied by the Medical Research Council.

REFERENCES

- Coste, F., Pierre-Bourgeois, Galmiche, P., Vic-Dupont, Mollon, R. H., Nahel, and Blatrix, C. (1951). *Rev. Tuberc.*, Paris, 15, 698.
 Israel, H. L., Sones, M., Stein, S. C., and Aronson, J. D. (1950). *Amer. Rev. Tuberc.*, 62, 408.
 Le Maistre, C. A., Tompsett, R., Muschenheim, C., Moore, J. A., and McDermott, W. (1951). *J. clin. Invest.*, 30, 445.
 Lemming, R. (1940). *Acta med. scand.*, 103, 400.
 Long, J. B., and Favours, C. B. (1950). *Johns Hopk. Hosp. Bull.*, 87, 186.
 Martenstein, H., and Jadassohn, W. (1923). *Klin. Wschr.*, 26, 1210.
 Sterling, K. (1950). *Amer. Rev. Tuberc.*, 62, 112.
 Vollmer, H. (1951). *J. Pediat.*, 39, 22.
 Wallner, L., Thompson, J. R., and Lichtenstein, M. R. (1952). *Amer. Rev. Tuberc.*, 66, 161.
 Wells, A. Q., and Wylie, J. A. H. (1949). *Lancet*, 1, 439.

TESTING OF SKIN DISINFECTANTS

BY

PETER STORY, M.D.

(From the Department of Pathology, St. Bartholomew's Hospital)

Skin disinfectants may be compared by their killing effect on normal skin flora or bacteria applied to the skin, and numerous techniques are based on this idea. Pathogenic bacteria are nearly always transient on the skin surface, where they can be killed by suitable chemicals, although in special circumstances pathogens such as *Staph. pyogenes* become established as part of the resident flora in deeper layers of the skin, where they may not be reached by disinfectants (Price, 1938). Normally there are resident bacteria deep in the skin which are mainly inaccessible to disinfectants or bacteriological sampling, but they are non-pathogenic organisms of little or no practical importance. Lovell (1945) has ingeniously demonstrated these deeply situated bacteria by sectioning and staining excised skin after a period of incubation in a moist chamber to allow organisms to multiply and become visible histologically.

Although there is general agreement that skin cannot be chemically sterilized throughout its thickness without destroying it, Gardner and Seddon (1946) have shown that the skin cocci can sometimes be eliminated completely. They excised and cultivated pieces of normal skin at the beginning and end of surgical operations; after preliminary treatment with 2% aqueous iodine 58%

yielded no growth of skin cocci; corresponding results with 5% and 1% aqueous cetrimide were 34% and 25%.

This last method could not be applied safely to skin contaminated with known pathogens, and to test a disinfectant's action against such organisms a practical method is to apply bacteria to the skin followed by disinfectant and recovery of any surviving bacteria after varying periods. This does at least supply the information that a disinfectant will destroy pathogenic bacteria known to be on the skin surface, where, indeed, they are most likely to be found under normal circumstances. Although open to the objection that bacteria placed on the skin may not be affected by disinfectants in the same way as natural transient flora, it is probably the best method of testing available.

In the investigation reported here this technique was used to test the efficacy of several skin disinfectants against strains of *Staph. pyogenes*, *Bact. coli*, *Ps. pyocyanea*, and *Proteus vulgaris* isolated from wound swabs. These organisms were all penicillin-resistant, and penicillin was added to the culture medium to suppress growth of normal skin flora. Most of the tests were carried out against a strain of *Staph. pyogenes* which grew freely in the presence of 0.5 unit of penicillin per ml. In experiments on quaternary ammonium compounds, such as cetrimide, the recovered organisms were mixed with sterile milk before cultivation, since it has been shown (McCulloch, 1947) that this substance will neutralize the bacteriostatic action of cetrimide. Bacteria were recovered from the skin by a method similar to Burtenshaw's (1938).

Numerous techniques have been employed in recovering bacteria from skin surfaces; they include rubbing the skin with a moist swab and then agitating the swab in broth and making pour plates (Colebrook, 1930) or streaking out the swab on an agar plate (Gardner and Seddon, 1946). Price (1938) devised the method of washing the hands and forearms in a series of bowls of water and cultivating this water; he has recently described a method for repeated bacteriological sampling of a small skin area which he calls "spot testing" (Price, 1951). Burtenshaw (1938) applied a rectangular glass chamber to the skin, added saline, and scraped the skin with a glass slide to make a suspension of organisms from which he made viable counts. Brown *et al.* (1944) filled watch-glasses to the brim with agar and placed them on skin to collect the bacteria. Most elaborate, perhaps, is the method of Nungester and Kempf (1942), who rubbed a mouse's tail with pneumococcal culture, dipped the tail into disinfectant for two minutes, and then cut off $\frac{1}{4}$ in. (1.3 cm.) and placed it in the mouse's peritoneal cavity. Any pneumococci surviving exposure to the disinfectant caused a fatal peritonitis.

Methods

For each disinfectant test six circular areas 4 cm. in diameter were outlined on the forearm or thigh, using a glass ring and skin pencil. Most tests were done on the forearm, the skin being unwashed. The bacterial inoculum to each test area consisted of 1 drop, from a pipette delivering 40 drops/ml., of a 1/10 dilution of 20-hour broth culture (in the case of *Ps. pyocyanea* only a 1/100 dilution was used). Each drop was spread over its circular area with a wire.

Bacteria were added to the first five circular areas. When dry the first four circles and the last (containing no added bacteria) were treated with disinfectant applied with a cotton-