Hospitals for their assistance in the examination and treatment of the children, and to Miss M. Hutchinson for considerable secretarial help.

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DEPRESSION OF TUBERCULIN SENSITIVITY IN GUINEA-PIGS BY CERTAIN ANTITUBERCULOUS SURFACE-ACTIVE AGENTS

BY

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Substances known to depress skin sensitivity to tuberculin are few in number (Long and Miles, 1950; Fisher, Harrington, and Long, 1951; Long, Miles, and Perry, 1951c), and when such depression is associated with anti-tuberculous action the question arises whether the chemotherapeutic mechanism is the same as, or is allied to, the anti-allergic.

The development of experimental tuberculosis in mice was shown by Cornforth, Hart, Rees, and Stock (1951) to be markedly inhibited by the injection of non-ionic, water-soluble, surface-active polyoxyethylene ethers of

$n=0, 1, 2, 3$, and higher integers. $x=10, 20$, or 60 (average value).

low toxicity, having the general formula given here, and obtained by polymerization of ethylene oxide with condensation products of formaldehyde with *p*-tert-octyl phenol. The antituberculous effect of these detergents

has since been confirmed in the guinea-pig, in which a substantial curative action was shown in an established (three-weeks-old) infection. Routine quantitative tuberculin tests made at the end of a 60-day course of treatment showed a marked reduction in hypersensitivity in the detergent-treated animals as compared with their controls. The desensitization appeared to be specific rather than due merely to suppression of tuberculosis, since it was more evident than in a parallel group of streptomycin-treated animals with somewhat greater suppression of disease. This unexpected observation has led us to a more precise study of the depression of tuberculin sensitivity by these agents in B.C.G.-sensitized guinea-pigs, using the method of Long and Miles (1950). We hoped to see whether this action resembled that of other substances known to diminish tuberculin allergy, and whether it was related to antituberculous activity.

Materials and Methods

Surface-active Agents.—Representative members of the series (see formula) were used (average of 20 units of ethylene oxide per phenolic nucleus, unless otherwise stated). These were D2, a mixture of compounds having various values of n from 0 upwards; D2a, a similar mixture but with a smaller proportion of the lower members of the series; D9a, an isolate with $n=1$ ("triplet"); D14, a mixture like D2 but with 10 units of ethylene oxide per phenolic nucleus, and therefore less hydrophilic; D22, a mixture like D2 but with an average of 60 units of ethylene oxide per phenolic nucleus, and therefore more hydrophilic. Compounds D2, D2a, and D14 were chemotherapeutically active in the tuberculous mouse; D9a and D22 were inactive. The compounds were synthesized by our colleagues, Dr. J. W. Cornforth and Mr. J. A. Stock. They were given intravenously in 0.5 ml., or subcutaneously in 1 ml., saline.

Cortisone was given as the acetate (Merck), subcutaneously in 1 ml. normal saline.

Guinea-pigs.—The Hampstead strain of albinos was used, the animals weighing about 350 g. They were fed on a pelleted diet (Bruce and Parkes, 1947) supplemented, unless otherwise stated, with unlimited cabbage. B.C.G.-sensitization was carried out as described by Long and Miles (1950).

Tuberculin Sensitivity.—This was tested by intracutaneous injections of 32 and 320 international units of O.T. in 0.2 ml. saline.

Effect on Tuberculin Sensitivity

The intravenous route was considered most likely to reveal any differences between the surface-active agents, since variations in absorption would be excluded. Intravenous injection of the chemotherapeutically active preparations D2, D2a, and D14 in doses of 10 mg. in the B.C.G.-sensitized guinea-pigs, immediately before tuberculin injections, produced a significant though limited diminution of sensitivity as compared with untreated sensitized controls; the diminution was of the same order as that produced by cortisone 2 mg. given subcutaneously six hours before the tuberculin. On the other hand, the chemotherapeutically inactive D9a and D22 were without appreciable effect in these intravenous doses, and even at 25 mg. (this dose, in the case of D22, being chosen so as to contain the same weight of phenolic nuclei as 10 mg. of D2—see above).

The desensitizing properties of D2a were further examined, choosing the more convenient subcutaneous route, injections being given in groups of ten B.C.G.-sensitized guinea-pigs two hours before the tuberculin. Under these conditions D2a gave its maximal effect on tuberculin depression, in comparison with the controls, at doses of 100 to 10 mg., there being no significant difference from the effect of cortisone 2 mg.; D2a had no effect at 1 mg.

Cortisone has no direct effect on tuberculin allergy; its desensitizing action depends on dietary factors (Long, Miles,

TABLE I.—Comparison of the Effect in Individual Treatment Groups with that in the Control Group (Same Experiment as Illustrated in Chart)

Treatment	Potency Ratio*	Variance Ratio†	P
Pelleted diet+			
D2a	0.23‡	20.28	<0.001
Cabbage and D2a	0.22‡	17.42	<0.001
Ascorbic acid and D2a	0.24‡	20.02	<0.001
Cabbage and propylthiouracil and D2a	0.14‡	41.99	<0.001
Cabbage and cortisone	0.18	22.49	<0.001

* Ratio of dose of tuberculin required to produce a reaction of given diameter in the controls (fed on pellets+cabbage), to corresponding dose in the treatment group.

† Ratio of "between treatments" variance to error variance.

‡ When cortisone was used instead of D2a the effect was not significant.

§ When cortisone was used instead of D2a the effect was significant.

and Perry, 1951a). It does not desensitize in guinea-pigs fed upon an ascorbic-acid-deficient diet, or on a diet supplemented with synthetic ascorbic acid, but only in animals provided with ascorbic acid in raw cabbage. Repetition of these experiments with D2a (10 mg. subcutaneously two

Dose response to 32 and 320 i.u. tuberculin in sensitive guinea-pigs. A=controls; B=ascorbic acid+D2a; C=D2a; D=cabbage+D2a; E=cabbage+cortisone; F=cabbage+propylthiouracil+D2a.

hours before tuberculin, instead of cortisone (2 mg.), in B.C.G.-sensitized animals showed that the desensitizing action of the detergent, unlike that of cortisone, was independent of all these factors (see Table I and Chart). Furthermore, the desensitizing action of cortisone in cabbage-fed guinea-pigs is prevented by propylthiouracil and restored by minimal doses of thyroxine (Long, Miles, and Perry, 1951b). On the other hand, propylthiouracil administered under similar conditions did not influence desensitization by D2a (see

Effect on Tuberculin Sensitivity and Antitoxic Immunity in the Same Animal

Three groups, each of ten guinea-pigs, were immunized by the subcutaneous injection into the left leg of 5 LF of diphtheria toxoid in 1 ml. normal saline; at the same time they were sensitized by the intramuscular injection into the right leg of 1.5 mg., dry weight, heat-killed virulent tubercle bacilli in 0.5 ml. liquid paraffin. Twenty-one days later the animals were immunized with a second dose of 5 LF diphtheria toxoid into the left leg. On the 23rd day one group was injected with 10 mg. of cortisone, a second with 50 mg. of D2a, while the third group, serving as controls, received 1 ml. normal saline alone; all injections were subcutaneous. The injections were repeated daily until the

31st day, when both flanks of the animals were depilated, and intracutaneous sensitivity to tuberculin estimated on the left side and to diphtheria toxin on the right side (Miles, 1949; Long, 1950). Both cortisone and D2a produced a comparable depression of tuberculin sensitivity, whereas neither influenced significantly skin sensitivity to diphtheria toxin in these immunized animals (see Table II).

All these animals were then bled by heart puncture, the serum was separated, and individual titrations of diphtheria antitoxin were carried out by the technique of Römer and Sames (1909). In addition, 1 ml. amounts of the sera of each of the three groups were pooled, and the antitoxin contents of the groups compared by passively immunizing guinea-pigs and testing their skin sensitivity to toxin, as described by Miles (1949). By neither method was it possible to detect differences in the antitoxin titre of the three groups. Thus it appears that under the conditions of this experiment neither cortisone nor D2a, in the heavy dosage employed, depressed antitoxin levels, nor did either prevent neutralization of intracutaneously injected toxin; nevertheless, both depressed tuberculin sensitivity to a similar degree, as previously observed.

Discussion

The present series of polyoxyethylene ethers represents a new type of antituberculous agent, with surface-active properties. Their mechanism is obscure, for, apart from producing submerged dispersed growth, they appear to have no direct action on the tubercle bacillus in the test-tube, even at 1% concentration, while there is no evidence of any antibacterial derivative in the blood taken from animals given large doses. Moreover, phagocytosis of tubercle bacilli is not increased, either *in vivo* or *in vitro*, by these agents (Rees and Hart, 1952).

The present work, by demonstrating a depression of skin sensitivity to tuberculin in the guinea-pig, shows that these surface-active agents have an effect on the host. This observation naturally invites comparison with other tuberculin-desensitizing substances, the most notable of which is cortisone (Long and Miles, 1950). The desensitizing effect of the present detergents is comparable in degree to that of cortisone, and, like the latter, does not affect antitoxic immunity in the guinea-pig; but there the analogy ends. For, first, the mechanism of the present effect is different, since it is independent of the dietary and hormonal influences that control the response to cortisone (cf. Long *et al.*, 1951a, 1951b, 1951c). Secondly, the detergents under study are antituberculous, whereas cortisone aggravates tuberculosis in mice (Hart and Rees, 1950). It is less certain that this steroid does so in the guinea-pig, which in resistance to cortisone, ascorbic acid metabolism, and ease of sensitization to allergens is more closely analogous to man than are the mouse and such creatures (Long, 1952). Nevertheless, dramatic effects in cortisone-sensitive animals, such as the mouse, rat, and rabbit, may indicate the trend of less obvious effects in cortisone-resistant creatures such as the guinea-pig and man.

The important role of cellular immunity, and of mononuclears in particular, in resistance to tuberculosis (Lurie, 1939a, 1939b, 1942) is virtually undisputed. On the other hand, repeated attempts to link humoral immunity and resistance to tuberculosis have failed (see, for example, Wilson and Miles, 1946). Cortisone causes lymphopenia and lymphoid atrophy in sensitive creatures (Wells and Kendall, 1940). Such effects are slight and inconstant in the guinea-pig, but, nevertheless, it is possible that cortisone-induced mesenchymal depression grossly outweighs the benefits of desensitization in cortisone-sensitive creatures, and cancels them in resistant ones. Thus only in animals treated with these detergents might anti-allergic activity exist without depression of the local cellular defence mechanism. This interpretation must remain hypothetical until further studies elucidate the problem.

However, we cannot overlook the most interesting observation that, whereas the chemotherapeutically active representatives of the present detergent series desensitized under the

TABLE II.—Comparison of the Effect in Individual Treatment Groups with that in the Control Group

Skin Response	Treatment	Potency Ratio*	Variance Ratio*	P
Bacterial allergy	Cortisone	0.33	23.19	<0.001
	D2a	0.46	18.39	<0.001
Antitoxic immunity	Cortisone	0.68	1.20	>0.2
	D2a	0.87	<1.0	—

* See Table I

conditions of our experiments, the inactive representatives did not. This association suggests that the mechanism of the chemotherapeutic action and that of the depression of sensitivity may have some common factor. Nevertheless, it must be admitted that there is at present little evidence in favour of the closer relationship of cause and effect—i.e., that the antituberculous effect is in fact due to desensitization. For although the many previous attempts to desensitize tuberculous guinea-pigs have had to be made with tuberculin, the results, taken as a whole, suggest little, if any, advantage to the host. Moreover, the present surface-active agents are equally effective in mouse and guinea-pig tuberculosis, in spite of the very different levels of allergy in these two species. Thus a tuberculin skin reaction cannot be elicited in the tuberculous mouse; and, although this animal can manifest a systemic reaction when tuberculin is given intravenously, much larger doses are required than in the guinea-pig (Smith, 1928; Rees and Hart, 1952). The influence on experimental tuberculosis of a number of other substances that depress tuberculin sensitivity, such as N-acetyl-sphingosine (Fisher *et al.*, 1951), dehydroascorbic acid and alloxan (Long *et al.*, 1951c), will be investigated. If these anti-allergic substances should also prove antituberculous, the hypothesis of a causal connexion between desensitization and chemotherapeutic activity would be strengthened.

Summary

The development of experimental tuberculosis in guinea-pigs is markedly inhibited by injection of certain surface-active polyoxyethylene ethers. Using the response to intracutaneous tuberculin as a measure of allergic hypersensitivity in albino guinea-pigs infected with B.C.G., we find that:

- (1) Single subcutaneous or intravenous injections of these drugs diminish sensitivity to a degree comparable to that of cortisone.
- (2) The chemotherapeutically active representatives of the series diminish tuberculin sensitivity, whereas the inactive members fail to do so.
- (3) Their mode of action differs from that of cortisone, since it is independent of dietary factors and of thyroid activity.
- (4) Neither D2a, a chemotherapeutically active detergent, nor cortisone, in heavy continual dosage, depressed skin sensitivity to diphtheria toxin, or antitoxin levels, in guinea-pigs immunized with diphtheria toxoid (in this experiment the animals were sensitized with dead tubercle bacilli). Both drugs depressed tuberculin sensitivity.
- (5) The possible mode of action of these surface-active agents is discussed.

We are indebted to Dr. Knud Tolderlund, of the State Serum Institute, Copenhagen, for the B.C.G.; to Dr. J. W. Cornforth and Mr. J. A. Stock for preparing the series of surface-active agents; and to Dr. W. L. M. Perry for the statistical analyses.

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TUBERCULOSIS IN FAMILIES WITH B.C.G.-VACCINATED AND NON- VACCINATED CHILDREN*

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Since the introduction of B.C.G. vaccination 25 years ago much has been written to prove or disprove its value. In the Scandinavian countries, in particular, investigators gradually became convinced that the Calmette-Guérin vaccine had a very important part to play in the prevention of primary tuberculosis in both children and adults.

Many workers all over the world have reported surveys demonstrating the protective value of the vaccine. Others found little evidence to support this view. The problem of carrying out controlled investigations to cover all possible variables has made it very difficult to assess its true value. The report of an outbreak of tuberculosis in a school in Denmark in 1943, investigated by Hyge (1947), fulfilled many of the conditions necessary for a true analysis of the protective value of B.C.G. vaccination. The reports of Heimbeck (1929, 1932), Rosenthal, Blahd, and Leslie (1945), and Aronson (1948) are examples of investigations with different types of control groups.

Another approach to the problem is to examine those families in whom some children have been vaccinated and others not. The number of reports of such families in the literature are few. In France, Bertoye (1936) described a family of four children. The second child was vaccinated at birth because it was customary to vaccinate all infants at the maternity hospital where the child was born. In June, 1935, a domestic servant had an unexpected haemoptysis. She was immediately removed to hospital and the diagnosis of tuberculosis confirmed. The following month the three non-vaccinated children (aged 8, 5, and 1½ years) each developed a fever with hilar adenitis and a positive tuberculin reaction. The youngest child later died of tuberculous meningitis. The vaccinated child (aged 6 years) remained quite well.

Selection of Families

A search was made in the records of the Norrtull's Hospital, the Samariten Hospital, and the Central Tuberculosis Dispensary, Stockholm, for families satisfying all the following conditions:

1. That some children in the family had been vaccinated with B.C.G. and some not.
2. That every child said to have been B.C.G.-vaccinated was actually vaccinated and had been negative to 1 mg. of old tuberculin at the time of vaccination. (All B.C.G. vaccinations and the post-vaccinal tests are notified to the Central Tuberculosis Dispensary, and the records were consulted.)
3. That the tuberculin reaction was positive at the time of the check test (at about three months in infants and six weeks in older children).
4. That the tuberculin test had become positive following the vaccination before the time of exposure to an active case of adult tuberculosis.

*This work was carried out at the Paediatric Clinic of the Karolinska Institute, Norrtull's Hospital, Stockholm, during the period of tenure of a Cow and Gate Travelling Scholarship from the Department of Child Health, Queen's University, Belfast.