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INFLUENCE OF CYSTEINAMINE ON TUBERCULIN SENSITIVITY IN GUINEA-PIGS

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As a result of a systematic search for a non-toxic substance protecting against the toxic effects of irradiation, Bacq and his colleagues focused their attention on cysteinamine $HS—CH_2—CH_2—NH_2$, the pharmacological actions of which have been recently reviewed (Bacq, Dechamps, Fischer, Herve, Le Bihan, Lecomte, Pirotte, and Payet, 1953). As might have been expected with an -SH compound (see, for example, Cronkite, Brecher, and Chapman, 1951 ; Ord and Stocken, 1953), mice were protected if the drug was injected immediately before, but not after, irradiation. Of greater significance, however, was the finding that if the liver was screened during irradiation, cysteinamine protected even when injected after irradiation. This delayed effect was the more remarkable because though initial damage was indistinguishable in cysteinamine treated and non-treated groups, resolution began three to four days later in cysteinamine treated but not in untreated animals. Moreover, cysteinamine relieved symptoms of irradiation sickness in man. This post-irradiation effect cannot be explained simply. Sulphydryl groups, though immediately effective (Cronkite *et al.*, 1951), do not show a delayed protection against irradiation. In addition, Bacq and his colleagues describe other properties of the drug that cannot be explained in terms of -SH activity ; cysteinamine exerts anti-mitotic activity on tissue cultures from chick embryos ; increased amounts of ascorbic acid and other reducing substances are present in plasma and urine of treated animals ; in addition, the Arthus phenomenon in rabbits, and tuberculin response in man, are inhibited by the drug (Bacq *et al.*, 1953).

Apparently an attempt was made to explain these rather contradictory findings by the hypothesis that cysteinamine behaves first like an -SH compound, and secondly as a " stressor agent " stimulating adrenocortical activity, for a series of experiments to test this hypothesis was carried out. Bacq and his colleagues (1953) found that, although the concentration of ascorbic acid in the adrenal cortex falls with cysteinamine treatment, this does not indicate increased adrenocortical activity, because levels of adrenocortical cholesterol, of corticoid excretion, and of circulating eosinophils stay constant.

However, even if cysteinamine does not increase adrenocortical activity, it might induce " cortisone-like " effects by acting as an anti-metabolite in an -SH system. It has been postulated that in the guinea-pig, cortisone interferes with the anabolism of protein resulting in a fall in tissue non-protein -SH, thus facilitating the oxidation of ascorbic to dehydroascorbic acid, the latter inhibiting phosphoglucomutase with the result that the concentration of glucose-1-phosphate in the tissues rises, this being associated with a diminished sensitivity to tuberculin (Cornforth and Long, 1953 ; Long and Spensley, 1954 ; Long, 1954). The initial step in this hypothetical chain of events is a decrease in tissue non-protein -SH. If the reaction involving -SH groups were blocked by cysteinamine or by one of its metabolites, the final effect might resemble that induced by cortisone.

The experiments described in this paper support this hypothesis. Cysteinamine has an immediate effect—like the -SH compound reduced glutathione —in preventing desensitization by cortisone. This effect can be attributed to its -SH group. It is followed by a delayed reverse effect, for cysteinamine injected six hours before the tuberculin depresses sensitivity. This action, like that of cortisone, is antagonized by reduced glutathione and does not occur in ascorbic acid deficient guinea-pigs (Cornforth and Long, 1953) ; at this stage cysteinamine neither assists nor antagonizes the desensitizing action of cortisone.

METHODS

Albino guinea-pigs of the Hampstead strain, weighing 350–550 g. and fed on a pelleted diet (Bruce and Parkes, 1947), supplemented (except in animals maintained in a state of ascorbic acid deficiency) with unlimited cabbage, were injected with B.C.G. vaccine, and sensitivity to tuberculin was estimated by the

method of Long and Miles (1950). Groups of 10 guinea-pigs were used in all experiments.

Reduced glutathione was dissolved in gas-free distilled water and adjusted with sodium bicarbonate solution to *p*H 6 ; approximately 150 mg./kg. body weight in a volume of 2 ml. was injected intraperitoneally two hours before the tuberculin. Cortisone acetate (5 mg./kg.) was suspended in 1 ml. of normal saline and injected six hours before the tuberculin. Cysteinamine (100 mg./kg.) adjusted to *p*H 7.5 with hydrochloric acid (Becaptan) was injected intraperitoneally at the time intervals indicated in each experiment.

As in previous work, the diameter of the tuberculin lesions, after 24 hr., was found to be proportional to the logarithm of the dose of tuberculin ; and it was possible to estimate the degree of sensitivity by the relative positions of the dosage–response lines fitted to the mean lesion-diameters plotted against the logarithm of the dose. Furthermore, an analysis of variance was calculated and showed the significance of effects. All differences quoted have been tested in this way and found to be significant at the P=0.001 level.

Results

Cysteinamine-Cortisone Relationships

Cortisone depressed sensitivity to tuberculin ; cysteinamine injected at the same time as the tuberculin did not influence sensitivity, but in cortisone-treated guinea-pigs it prevented desensitization

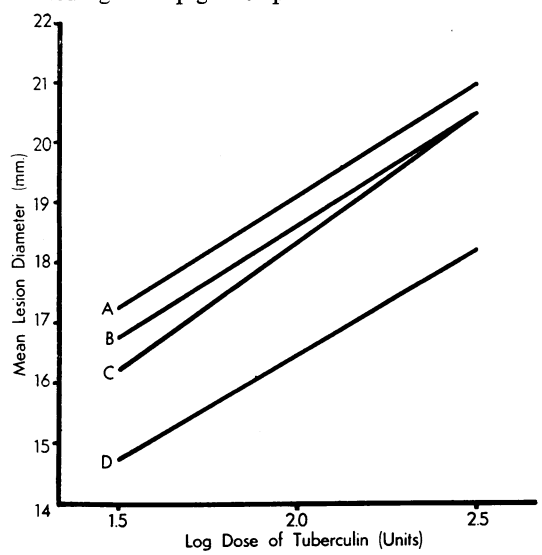

FIG. 1.—Response to 32 and 320 i.u. (International Units) tuberculin in sensitive guinea-pigs. A, Controls. B, Cysteinamine injected at same time as tuberculin. C, Cysteinamine injected at same time as tuberculin+cortisone injected six hours beforehand. D, Cortisone injected six hours before tuberculin.

(Fig. 1). Cortisone and cysteinamine both depressed sensitivity when injected six hours before the tuberculin, and this was true whether they were injected alone or together (Fig. 2).

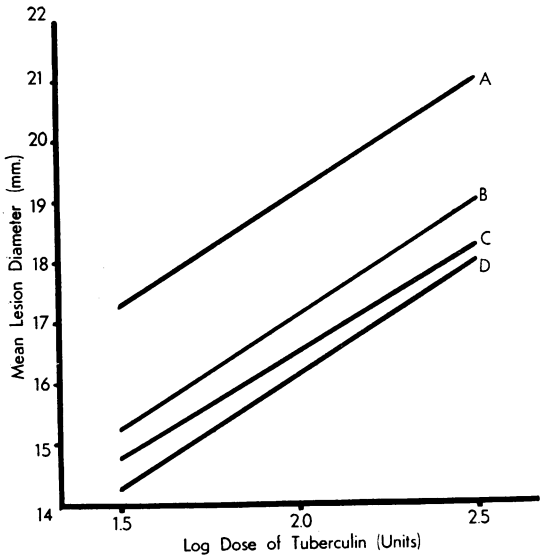

FIG. 2.—Response to 32 and 320 i.u. tuberculin in sensitive guinea-pigs. A, Controls. B, Cysteinamine injected six hours before the tuberculin. C, Cortisone injected six hours before the tuberculin. D, Cortisone+cysteinamine both injected six hours before the tuberculin.

Cysteinamine-Glutathione-Ascorbic Acid Relationships

Reduced glutathione injected at the same time as the tuberculin did not influence sensitivity, but prevented desensitization induced by cysteinamine injected six hours beforehand (Fig. 3). Cysteinamine did not influence sensitivity in ascorbic acid deficient guinea-pigs (Fig. 4).

Discussion

Cysteinamine, in the tuberculin sensitive guinea-pig, behaves first like an -SH compound ; this effect is in keeping with its protective power in mice when injected immediately before irradiation (Bacq *et al.*, 1953). This brief action is succeeded by a cortisone-like effect on sensitivity to tuberculin, which is antagonized by an -SH compound and does not occur in ascorbic acid deficient guinea-pigs (cf. Cornforth and Long, 1953)—an effect in keeping with the anti-mitotic and anti-allergic activity of the drug (Bacq *et al.*, 1953). This cortisone-like action, as already stated, cannot be attributed to increased adrenocortical activity (Bacq *et al.*, 1953). It is suggested that cysteinamine blocks the metabolism of -SH compounds after the manner of an anti-metabolite, and, as a result, the level of ascorbic acid and of other reducing substances in plasma and urine increases as described by Bacq. Further support for this hypothesis is obtained from the fact that

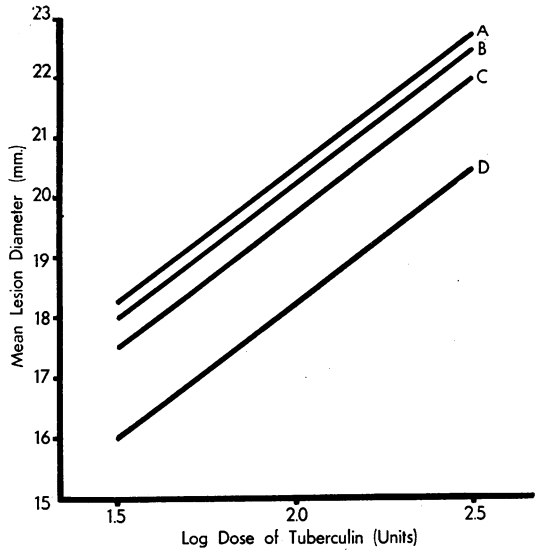

FIG. 3. — Response to 32 and 320 i.u. tuberculin in sensitive guinea-pigs. A, Reduced glutathione. B, Controls. C, Cysteinamine + reduced glutathione. D, Cysteinamine.

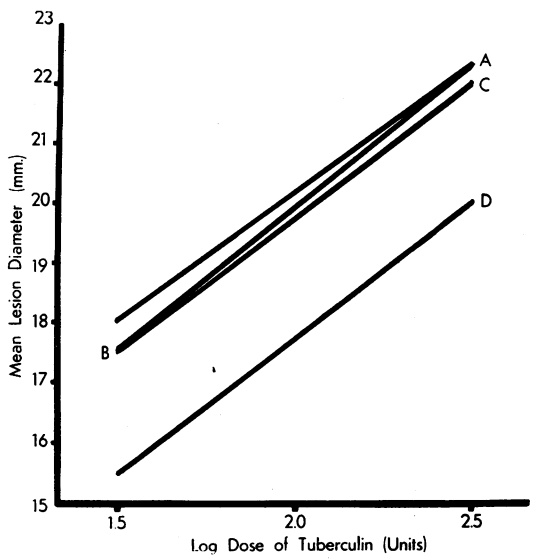

FIG. 4. — Response to 32 and 320 i.u. tuberculin in sensitive guinea-pigs. A, Cysteinamine. B, Ascorbic acid (provided as cabbage). C, Controls. D, Cysteinamine + ascorbic acid (provided as cabbage).

cortisone-induced hepatic hyperactivity is associated in many different species, including the guinea-pig, with hypertrophy of the liver (Lloyd and Long, 1953); whereas, in direct contrast, treatment with cysteinamine causes atrophy of the liver (Bacq *et al.*, 1953). Atrophy is commonly attributed to decreased activity, which, according to the hypothesis now advanced, can be attributed to a block of metabolism.

SUMMARY

1. Using the response to intradermal tuberculin as a measure of allergic hypersensitivity in albino guinea-pigs injected with B.C.G., it was found that:

(*a*) A single intraperitoneal injection of cysteinamine, given at the same time as the tuberculin, prevented desensitization by cortisone.

(*b*) A single intraperitoneal injection of cysteinamine, given six hours before the tuberculin, depressed sensitivity to tuberculin, and this action occurred in the presence or absence of cortisone.

(*c*) Desensitization produced by a single intraperitoneal injection of cysteinamine was prevented by reduced glutathione.

(*d*) Desensitization produced by a single injection of cysteinamine did not occur in ascorbic acid deficient guinea-pigs.

2. It is concluded that cysteinamine has two actions in the guinea-pig, an immediate action in which it behaves like an -SH compound, followed by a more prolonged action, in which it produces effects analogous to those of cortisone. It is suggested that the latter effects may be due to cysteinamine or one of its breakdown products behaving like an anti-metabolite.

I am grateful to Professor Z. M. Bacq for drawing my attention to the action of this interesting drug and for providing me with an advance copy of his recent review before publication.

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