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INFLUENCE OF ORGANIC PHOSPHATES ON TUBERCULIN SENSITIVITY IN B.C.G. INFECTED GUINEAPIGS

RELATION TO CORTISONE DESENSITISATION

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A.C.T.H. and cortisone depress, and thyroxine increases, hypersensitivity to tuberculin in guineapigs infected with B.C.G. (Long and Miles 1950). But small amounts of thyroxine are needed for desensitisation by the adrenal hormones, which then depress hypersensitivity to the same final level irrespective of the amount of thyroxine available (Long, Miles, and Perry 1951b). The link between this complex interaction of hormones may lie in the metabolism of ascorbic acid; for it is probable that, in the guineapig, the oxidation of ascorbic acid to dehydroascorbic acid initiates desensitisation, and that the thyroid and adrenal hormones exert their influence on the sulphydryl reducing system responsible for maintaining in the tissues the balance between ascorbic and dehydroascorbic acid (Long, Miles, and Perry 1951a and c, Long 1952). This argument is strengthened by the observation that alloxan, which combines with -SH compounds, diminishes sensitivity to tuberculin and bears the same relation to dietary and hormonal factors as cortisone (fig. 1) (Long et al. 1951a, b, and c); and also by the fact that the injection of the -SH compound glutathione prevents desensitisation by cortisone (Long et al. 1951c).

In vitro, alloxan inhibits the conversion of glucose-1-phosphate (Cori ester) to glucose-6-phosphate (Robinson ester), the second step in the metabolism of glycogen. This inhibition is attributed to its action as an enzyme destroyer (Lehmann 1939). Alloxan, which has in any case a very short life in the body (Archibald 1945), may not itself exert this influence on the enzyme in vivo, but may do so by inducing overproduction of dehydroascorbic acid, which has already been inferred to be the more immediate agent in alloxan desensitisation (Long et al. 1951c) and which has a chemical structure similar to alloxan. If, then, these speculations are correct, cortisone and alloxan facilitate the formation of dehydroascorbic acid which inactivates phosphoglucomutase and so blocks the conversion of glucose-1-phosphate to glucose-6-phosphate, with accumulation of the former (fig. 2). On the assumption that glucose-1-phosphate represents the next step in the chain of metabolic events leading to diminished sensitivity to tuberculin, it was tested for desensitising power and proved active in a dose of less than 0·025 mg. per kg. body-weight, which is many times less than the minimum effective dose of cortisone, alloxan, or dehydroascorbic acid. Glucose-1-phosphate is of course normally present in the body, but its presence has not to our knowledge been reported in extracellular fluids in vivo. This may account for the effect observed on injection of a dose which must be quite small relative to the body's normal turnover of glucose-1-phosphate. Glucose-6-phosphate, the product formed from glucose-1-phosphate by phosphoglucomutase was also found to desensitise, but the dose required is several hundred times larger than the effective dose of glucose-1-phosphate. This effect may perhaps be due to the equilibrium glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate, a large excess of the latter component producing a smaller excess of the former.

In the present paper, the effect on tuberculin sensitivity of glucose-1-phosphate and of other biologically significant phosphorus compounds is compared with that of other desensitising agents—namely, cortisone, alloxan, ascorbic acid and its metabolites (Long 1952), N-acetylsphingosine (Fisher, Harington, and Long 1951), and certain phenolic polyoxyethylene ethers (D'Arcy Hart, Long, and Rees 1952). In addition, the observation that lysergic acid diethylamide, an amide of one of the constituents of ergot, increases, in very small oral doses, the level of hexosemonophosphate in the blood of man, apparently by blocking its further enzymatic breakdown (Mayer-Gross, McAdam, and Walker 1951), provided a second method for testing our hypothesis, since if our speculations are correct, this drug should cause a diminution in sensitivity to tuberculin. It did so, in a dose of less than 0·0002 mg. per kg.

METHODS AND RESULTS

350 g. albino guineapigs of the Hampstead strain, fed upon a pelleted diet (Bruce and Parkes 1947) supplemented with unlimited cabbage, were injected with B.C.G. vaccine, and sensitivity to tuberculin was estimated by the method of Long and Miles (1950). All drugs tested, with the exception of glutathione, N-acetylsphingosine, and cortisone acetate, were dissolved in 1 ml. of normal saline and injected subcutaneously two hours before the tuberculin.

Phosphates supplied as barium salts were dissolved in saline, treated with a little sodium sulphate, and freed from barium sulphate before injection. Where the substance was given as a salt, the dose has been recalculated to give the amount of organic anion actually present. N-acetylsphingosine and cortisone acetate were suspended in 1 ml.

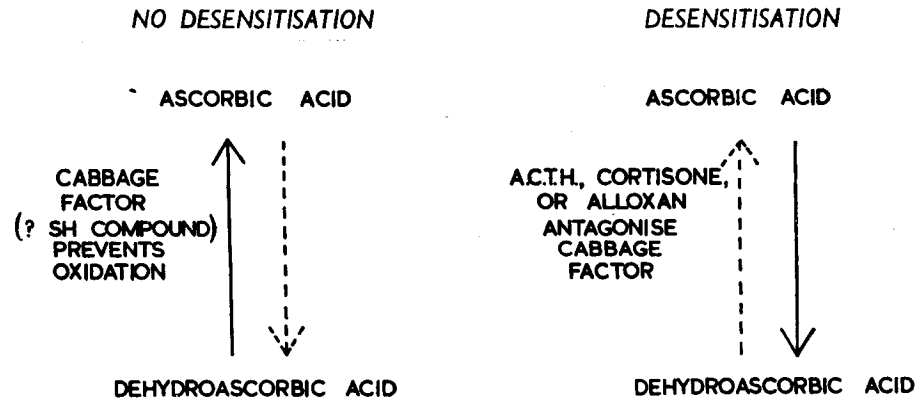

Fig. 1.

of normal saline and injected subcutaneously six hours before the tuberculin. Reduced glutathione was dissolved in gas-free distilled water and adjusted with sodium bicarbonate solution to pH 6; approximately 150 mg. per kg. in a volume of 2 ml. was injected intraperitoneally two hours before the tuberculin.

The dose of a drug depended partly on its availability: some phosphates, inactive in the dose actually given, might at higher dose levels have desensitised.

After preliminary experiments had shown glucose-1-phosphate to be an effective desensitising agent in doses of 0·01 mg. per kg., whereas glucose (100 mg. per kg.) and sodium phosphate (100 mg. per kg.) were without effect, it was decided to test, on an empirical basis, other organic phosphate compounds representing the various stages of phosphorus metabolism. Glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-diphosphate, though active in a dose of approximately 50 mg. per kg, were inactive at 3 mg. per kg. Fructose-1-phosphate (3 mg. per kg.), phosphopyruvate (2·5 mg. per kg.), phosphoglycerate (2·5 mg. per kg.), riboflavine phosphate (15 mg. per kg.), adenosine triphosphate (12 mg. per kg.), adenosine-5′-phosphate (50 mg. per kg.), and glycerophosphate (50 mg. per kg.) were without significant effect. Ribose-5-phosphate had comparable desensitising power to glucose-1-phosphate, being effective in a dose of 0·04 mg. per kg. Adenosine diphosphate (2·5 mg. per kg.) and adenosine-3′-phosphate (2·5 mg. per kg.) proved effective. Lysergic acid diethylamide, as already stated, diminished sensitivity to tuberculin in a dose of 0·00018 mg. per kg.

According to our hypothesis, the activity of phosphoglucomutase is inhibited by dehydroascorbic acid, with the result that glucose-1-phosphate accumulates and causes a diminution in sensitivity to tuberculin. But reduced glutathione assists the action of phosphoglucomutase (Lehmann 1939), so that dehydroascorbic acid might block phosphorylation by oxidising glutathione rather than by exerting an anti-enzymic effect. If so, the injection of an excess of reduced glutathione should prevent desensitisation by glucose-1-phosphate. It failed to do so (see table), thus supporting the idea that dehydroascorbic acid influences phosphoglucomutase itself. Reduced glutatione prevents desensitisation by cortisone acetate and by ascorbic acid (Long et al. 1951b); this was confirmed and shown also to apply to desensitisation by alloxan and dehydroascorbic acid, but not to desensitisation by glucose-1-phosphate, the phenolic polyoxyethylene ethers or N-acetylsphingosine. This suggests, first that -SH compounds can block the chain of chemical events as far as dehydroascorbic acid, and secondly that N-acetylsphingosine and the polyoxyethylene ethers exert their effect after this stage. They may in fact influence sensitivity by affecting phosphorus metabolism, with which sphingosine is known to be linked, but we have no evidence to support this contention. Neither are we in a position to explain the marked desensitising properties of ribose-5-phosphate or the less marked effects of adenosine diphosphate or adenosine-3′-phosphate. Nevertheless, the independence on dietary and hormonal factors of the action of glucose-1-phosphate, the lack of antagonistic effect exerted by glutathione, the very small effective dose and the confirmatory evidence provided by the desensitising action of lysergic acid diethylamide, suggest to us that glucose-1-phosphate is more directly linked with the sensitivity of tissues to tuberculin than any other substance with which we have experience.

EFFECT OF REDUCED GLUTATHIONE UPON DESENSITISATION BY VARIOUS AGENTS

Agent and dose per kg. body-weight	Desensitisation	
	Glutathione (150 mg. per kg.)	No glutathione
Alloxan (25 mg.)	−	+
Ascorbic acid * (50 mg.)	−	+
Cortisone acetate (5 mg.)	−	+
Dehydroascorbic acid (50 mg.)	−	+
Glucose-1-phosphate (12 mg.)	+	+
N-acetylsphingosine (30 mg.)	+	+
Polyoxyethylene ethers (20 mg.)	+	+

* In pellet-fed guineapigs.

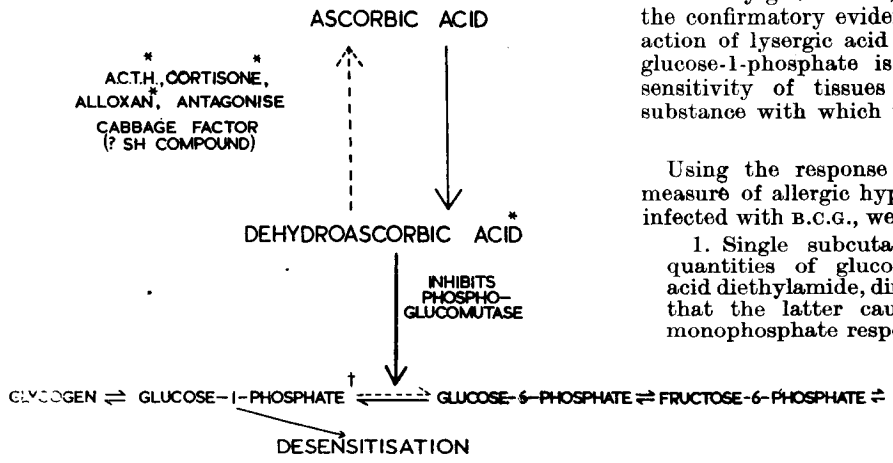

Fig. 2.

* Desensitising action prevented by reduced glutathione.
† Desensitising action not prevented by reduced glutathione.

SUMMARY

Using the response to intradermal tuberculin as a measure of allergic hypersensitivity in albino guineapigs infected with B.C.G., we find that:

1. Single subcutaneous injections of very small quantities of glucose-1-phosphate, and of lysergic acid diethylamide, diminish sensitivity. It is suggested that the latter causes an accumulation of hexose monophosphate responsible for densensitisation.

2. Injected reduced glutathione prevents the desensitising action of cortisone acetate, alloxan, ascorbic acid, and dehydroascorbic acid, but not of glucose-1-phosphate, N-acetylsphingosine and the polyoxyethylene ethers. The

relation of these findings to the mechanism of cortisone desensitisation are discussed.

Our present hypothesis (see fig. 2) provides what we believe to be a useful, though probably oversimplified, summary of the mechanism of cortisone desensitisation. We suggest that cortisone facilitates the oxidation of ascorbic to dehydroascorbic acid. Dehydroascorbic acid inhibits phosphoglucomutase, and as a result glucose-1-phosphate accumulates in the tissues and is intimately concerned with desensitisation.

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