The Influence of Bacterial Exotoxins on the Non-Protein -SH Content of the Tissues of Different Animal Species

BY JULIA GERWING AND D. A. LONG

National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 18 April 1958)

Cortisone acetate (Long & Miles, 1950) and hydrocortisone (Long & Spensley, 1954) depress sensitivity to tuberculin in guinea pigs. This desensitization does not occur in guinea pigs mildly deficient in ascorbic acid, and is thought to be associated with the oxidation of ascorbic acid to dehydroascorbic acid. There appears to exist an antagonism between corticosteroid desensitizing agents and glutathione in that injection of reduced glutathione prevents desensitization in all cases (Long, 1954). From these findings it has been suggested that adrenocortical activity is intimately concerned in -SH metabolism.

There is evidence that adrenocortical stimulation or the injection of corticotrophin or of cortisone acetate decreases blood non-protein -SH. Cortisone has been shown to cause a decrease in blood glutathione in the rat proportional to the glycosuria produced (Lazarow & Berman, 1950). Several workers have shown that in man, cortisone treatment results in decreased blood glutathione proportional to the increase in blood sugar (Hess, Kyle & Doolan, 1951; Goldzeiher, Epstein, Rawls & Goldzeiher, 1953; Kass, Ingbar & Finland, 1950). In agreement with this, Conn and his colleagues (Conn, Louis & Wheeler, 1948; Conn, Louis & Johnston, 1949*a, b*) have shown that corticotrophin induced hyperglycaemia and a fall in blood non-protein -SH in man, and that pre-treatment with glutathione alleviated the hyperglycaemia.

METHODS

In order to exclude variations in endocrine activity due to the oestrous cycle, male animals were used throughout. The species and approximate weights were: albino mice of the Parkes strain, 20 g.; hooded rats, 180 g.; albino guinea pigs of the Hampstead strain, 450 g.; sandy half lop-eared rabbits, 2.0 kg.; Dutch rabbits, 1.5 kg.; Rhesus monkeys, 2.5-3.0 kg. Mice and rats were fed on Bruce & Parkes (1949) pelleted diet 41; guinea pigs and rabbits on Bruce & Parkes (1947) pelleted diet 18, supplemented with 5% of skimmed milk, hay and cabbage. To examine the effect of diet on the responses of rabbits, a second group was fed from the time of weaning on a diet of oats, fishmeal, hay and cabbage. The Rhesus monkeys were given diet 41 supplemented with cabbage and dates. All animals had tap water to drink and were maintained in temperature-controlled rooms at 18-20°.

The bacterial exotoxins

Three preparations of bacterial exotoxin, with widely different pharmacological actions, were selected:

Corynebacterium diphtheriae toxin. T.P. 2776 (produced by the Wellcome Research Laboratories), the current laboratory standard diphtheria toxin, was used, Lr/1000 dose = 0.02 μ l. (Wilson & Miles, 1955); international Schick unit = 0.03 μ l. (as assayed by the method of Gerwing, Long & Mussett, 1957). The dose for guinea pigs was 30 i.u. into the adductor muscles of the left leg.

Shigella shigae (*dysentery toxin*) (toxin BI). The current laboratory standard 'Shiga' toxin was used: LD₅₀ for rats, 0.45 ml.; LD₅₀ for guinea pigs, 1.0 ml. The dose for rats was 0.40 ml. intraperitoneally, and for guinea pigs 0.25 ml. intraperitoneally.

Clostridium septicum toxin (V.S. VI). The current laboratory standard toxin was used. This toxin has the advantage that all the species included in this paper are susceptible to its toxic action. The L+ (Wilson & Miles, 1955) dose for a mouse is 2.6 mg.

All species were injected with 50 mg./kg. into the adductor muscles of the left leg. A further experiment was carried out in rabbits, in which the effects of 50, 10 and 2 mg. of toxin/kg. were compared.

Determination of non-protein -SH

The method of Grunert & Phillips (1951) was used with a minor modification. The final step of this method involved the addition first of a nitroprusside reagent and then of a sodium cyanide-sodium carbonate mixture to the tissue filtrate. The colour obtained is proportional to the non-protein -SH content of the filtrate. The acid filtrate rapidly destroyed the nitroprusside and made consistent results hard to obtain, but this could be prevented by adding the alkaline mixture immediately before the nitroprusside. Direct experiments with known dilutions of glutathione confirmed the validity of this modification. Intensity of colour was measured in a Unicam SP. 350 absorptiometer at a wavelength of 520 μ .

RESULTS

Rat. Forty rats were injected with *Cl. septicum* toxin. Ten toxic and ten non-toxic controls were killed with chloroform at intervals of 24 hr. Samples of the middle lobe of the liver and of the adductor muscle of the right front leg were taken, weighed and frozen in dry ice. The non-protein -SH content of samples was calculated as mg./100 g. of tissue.

The maximal effect was obtained after 24 hr.; muscle non-protein -SH levels were 36.6 % lower in toxic than in non-toxic rats; liver levels had increased by 54.2 %. Gradual recovery occurred with time (Figs. 1-3).

A comparable statistically significant effect was produced with *Sh. shigae* toxin after 24 hr. (Table 1).

Fig. 1. Effect of *Cl. septicum* toxin on the levels of non-protein -SH in the liver (—) and muscle (- - -) of rats. (Differences between control and stressed livers: 24 hr. $P < 0.001$; 48 hr. $P = 0.01-0.001$; 72 hr. and 96 hr. $P > 0.1$. Differences between control and stressed muscle: 24 hr. $P < 0.001$; 48 and 72 hr. $P = 0.01-0.001$; 96 hr. $P = 0.05-0.02$.)

Mouse. Comparable experiments with *Cl. septicum* toxin injected into ten mice produced effects similar to those obtained in rats (Figs. 2, 3).

Guinea pig. Forty guinea pigs were injected with *Cl. septicum* toxin and an experiment was carried out which was essentially the same as the first described for rats. The non-protein -SH content of the livers of animals treated with toxin were not altered significantly. A significant fall in the non-protein -SH content of muscle occurred at 24 hr. and this was followed by rises above normal of borderline significance. However, the fact that this increase occurred on successive days suggests that the effect is real, though slight (Figs. 2-4).

Comparable effects were obtained with *Sh. shigae* toxin and with *C. diphtheriae* toxin.

Monkey. Five Rhesus monkeys were injected into the adductor muscle of the left hind leg with *Cl. septicum* toxin. Significant decreases of non-protein -SH levels were found in both liver and muscle (Figs. 2, 3).

Rabbit. The rabbits in this Laboratory are very susceptible to agents that damage the liver, and even in normal control animals the incidence of cirrhosis of the liver is high. As little as 100 i.u. of diphtheria toxin killed rabbits of 2.0-1.5 kg. Death was associated with marked liver damage. In such animals the non-protein -SH of the liver fell and the level in muscle rose. Because of these findings, and of a long-standing belief that the diet of rabbits contributed to their liver disease, Dutch rabbits were taken at the time of weaning and fed on a new full diet. This strain of rabbit was selected because it had always thrived better than the other strains kept in this Institute. An experiment was carried out on them, when mature, in which graded doses of toxin were given to different groups. The results (Fig. 5) showed that the smallest amount of *Cl. septicum* toxin tested (2 mg./kg.) gave results comparable with those obtained in the

Table 1. Effect at 24 hr. of toxin on the non-protein -SH levels of liver and muscle tissues

Animal	Tissue	Toxin	Non-protein -SH (mg./100 g. of tissue)	Degrees of freedom	<i>t</i>	<i>P</i>
Rat	Control liver		163.5	18	4.62	<0.001
	Stressed liver	<i>Sh. shigae</i>	177.5			
	Control muscle		17.0	18	5.65	<0.001
	Stressed muscle	<i>Sh. shigae</i>	11.6			
Guinea pig	Control liver		267.8	18	1.21	>0.1
	Stressed liver	<i>Sh. shigae</i> *	261.8			
	Control muscle		28.7	18	5.13	<0.001
	Stressed muscle	<i>Sh. shigae</i> *	22.0			
Guinea pig	Control liver		266.5	18	1.06	>0.1
	Stressed liver	<i>C. diphtheriae</i> *	259.5			
	Control muscle		27.7	18	4.36	<0.001
	Stressed muscle	<i>C. diphtheriae</i> *	22.6			

* Single injection.

Fig. 2. Effect at 24 hr. of a single injection of *Cl. septicum* toxin on non-protein -SH levels of liver in various animal species. (Analyses of differences: mice, $P < 0.001$; rats, $P < 0.001$; guinea pigs, $P > 0.1$; monkeys, $P < 0.001$.) □, Control; ■, stress.

Fig. 3. Effect at 24 hr. of a single injection of *Cl. septicum* toxin on muscle non-protein -SH in various animal species. (Analyses of differences: mice, $P < 0.001$; rats, $P < 0.001$; guinea pigs, $P < 0.001$; monkeys, $P < 0.001$.) □, Control; ■, stress.

rat and mouse, namely an increase in the non-protein -SH content of liver and a decrease in the amount present in muscle; but, apparently, the new diet did not significantly increase their resistance to bacterial exotoxins.

Fig. 4. Effect of a single injection of *Cl. septicum* toxin on levels of non-protein -SH in liver (—) and muscle (- - -) of guinea pigs.

Fig. 5. Effect at 24 hr. of various doses of *Cl. septicum* toxin on liver (—) and muscle (- - -) non-protein -SH in rabbits. (Analyses of differences in liver tissues: 50 mg. of toxin/kg. $P < 0.001$; 10 mg./kg. $P > 0.1$; 2 mg./kg. $P = 0.01$. Analyses of differences in muscle tissues: 50 mg. of toxin/kg. $P > 0.1$; 10 mg./kg. $P = 0.5-0.01$, 2 mg./kg. $P = 0.01$.)

DISCUSSION

In all the species tested, bacterial exotoxins cause a fall in the non-protein -SH content of muscle. Their effect on the non-protein -SH content of liver is variable. In the rat, mouse and rabbit, liver levels of non-protein -SH increased when these species were injected with toxins. In the guinea pig the liver was unaffected and, in the Rhesus monkey, there was a decrease in liver non-protein -SH. It has previously been noted that a profound species difference exists in the response of the rat, rabbit and mouse, on one hand, and the guinea pig and the Rhesus monkey, on the other, to prolonged cortisone treatment. The former group lose body weight and show a fall in circulating γ -globulin, whereas the latter group maintain body weight and circulating γ -globulin (Shewell & Long, 1956). This species difference is maintained in the response of the thyroid glands of these animals to intoxication. Bacterial exotoxins stimulate activity in both the thyroid gland and the adrenal cortex in guinea pigs and Rhesus monkeys, but cause stimulation of the adrenal cortex with a reciprocal depression in thyroid activity in rats, mice and rabbits (Gerwing, Long & Pitt-Rivers, 1958). It is possible that this difference in the endocrine response to toxæmia is responsible in some way for the differences in the effect of toxic stress on liver non-protein -SH in these species. However, the outstanding fact remains that stress produced by this means causes a fall in non-protein -SH in the muscle of all species tested.

As indicated above, there is a great deal of indirect evidence to suggest that corticotrophin and cortisone depress sensitivity to tuberculin in the skin of the guinea pig by influencing -SH metabolism (Cornforth & Long, 1952, 1953; Long, 1954, 1955). These findings gave rise to the hypothesis that cortisone (Cornforth & Long, 1953) interfered with the metabolism of glutathione in the course of a general interference with protein synthesis. If this hypothesis is true, one might expect to find a subnormal level of non-protein -SH in the carcass of animals under stress. The hypothesis is supported to the extent that this effect has now been found.

SUMMARY

1. A single injection of bacterial exotoxin causes an increase in liver non-protein -SH and a decrease in muscle non-protein -SH in rats and mice.

2. A single injection of toxin did not affect the level of non-protein -SH in the liver of guinea pigs but caused a decrease in the level in muscle.

3. A single injection of toxin caused a decrease in the levels of non-protein -SH in both liver and muscle of Rhesus monkeys.

4. A single injection of a small dose of toxin caused an increase in the level of non-protein -SH in the liver and a decrease of the level in muscle of rabbits. Large doses of toxin caused liver damage associated with reverse effects.

5. The importance of considering difference between species is emphasized.

6. The importance of considering differences between the response to stress of the viscera and of the carcass is emphasized.

The authors are most grateful to Dr J. W. Cornforth, F.R.S., for his help and advice in this work. This work forms part of a Thesis (J.G.) accepted by the University of London in part fulfilment of the requirements for the degree of Doctor of Philosophy.

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