A NOTE ON BACTERICIDES IN SOLUTIONS FOR INJECTION

By H. B. SWIFT.

Department of Pharmacology, Amritsar, India
From the Department of Pharmacology, University of Manchester

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ONE of the methods described in the British Pharmacopœia for sterilising aqueous solutions is "heating with a bactericide." The bactericides recommended are 0·2 per cent. solution of chlorocresol (*p*-chloro-*m*-cresol) or 0·002 per cent. solution of phenylmercuric nitrate. Davis and Davison[1] have shown by means of the filtration sterility test that relatively low concentrations of bacterial spores may survive after heating with 0·2 per cent. solution of chlorocresol. In the discussion on this paper, Hartley[2] pointed out that chlorocresol in concentrations of 0·2 per cent. or less was inadequate for sterilisation and asked the authors to suggest a more suitable concentration.

Wien[3] determined the chronic toxicity of 0·25 per cent. solution of chlorocresol and of 0·002 per cent. solution of phenylmercuric nitrate by experiments on rabbits. The purpose of the experiments described in this paper was to determine the chronic toxicity of higher concentrations so that if the present concentrations are found inadequate, appropriate concentrations may be recommended.

CHLOROCRESOL

Chronic Toxicity as determined by experiments on rabbits. In the following experiments 0·4 per cent solution in distilled water, prepared by dissolving in hot water and cooling to room temperature, was used. The experiment was made on two rabbits. Each received daily 5 ml. of the 0·4 per cent solution subcutaneously. A third rabbit used as control received daily 5 ml. of distilled water subcutaneously. The urine was examined every other day by qualitative tests for albumen and blood. Once a week the urine was examined for deposit of epithelial cells or casts. All the results were negative. The blood was examined once a week and no pathological abnormalities were found in the red or white cell counts. The variations in the white cell count were the same as in the control animal. Values are given for each week in Table I. The appearance of the rabbits remained normal in every way. There was no diarrhœa except in the case of the control rabbit which recovered in a day with careful feeding.

Histological examination of tissues. The liver and kidneys were examined. Sections were stained with eosin. Microscopic examination showed that all the specimens were normal. The skin at the site of injection showed no degenerative changes.

Effect of intracisternal injection on the pressure and cell content of the cerebrospinal fluid. The general experimental procedure was similar to that described in the literature (Bedford[3,4]). 1 ml. of 4 per cent. solution was introduced into the cisterna magna of three dogs. The animals, after recovery from the anæsthetic, were allowed to survive for 6 hours. The results are shown in Table III.

TABLE I

CHLOROCRESOL SUBCUTANEOUSLY IN RABBITS

RABBIT I (CONTROL)

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	1·75	1·65	1·65	1·75
Hæmoglobin, per cent.	92	90	86	82
Red cells /cu.mm	6,000,000	6,000,000	6,350,000	6,300,000
Colour index	0·8	0·75	0·68	0·65
White cells cu./mm	9,000	7,200	15,000	9,400
Lymphocytes, per cent.	68	70	67	70
Granulocytes, per cent.	32	30	33	30

RABBIT II

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	1·8	1·75	1·8	1·7
Hæmoglobin, per cent.	95	88	90	81
Red cells, cu./mm	6,430,000	6,350,000	6,750,000	6,550,000
Colour index	0·74	0·59	0·67	0·6
White cells /cu.mm	11,000	14,000	11,600	8,800
Lymphocytes, per cent.	66	52	55	66
Granulocytes, per cent.	34	48	45	34

RABBIT III

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	2·0	2·1	2·1	2·15
Hæmoglobin, per cent.	85	75	73	73
Red cells /cu.mm	6,650,000	5,900,000	6,500,000	5,650,000
Colour index	0·67	0·64	0·57	0·65
White cells /cu.mm	14,000	13,800	8,600	9,600
Lymphocytes, per cent.	68	67	67	70
Granulocytes, per cent.	32	33	33	30

PHENYLMERCURIC NITRATE

The basic salt $C_6H_5HgNo_3C_6H_5Hg(OH)$ was used. A 0·004 per cent. solution was obtained by dissolving in hot distilled water and cooling to room temperature.

Chronic toxicity as determined by experiments on rabbits. Two rabbits were used. Each received daily for 4 weeks 5 ml. of the solution subcutaneously. A third rabbit was used as control and received daily 5 ml. of distilled water subcutaneously. The urine was examined every day for albumen and blood and once a week for deposits or casts. All the results were negative. Blood examinations are shown in Table II.

Pathological examination of the tissues. The kidney and liver, and the skin at the site of the injections were examined. There were no degenerative changes.

Effect of intracisternal injection on the pressure and cell content of the cerebrospinal fluid. Two dogs were used for this experiment. Results are comparable with those with chlorocresol and are shown in Table III.

TABLE II

PHENYLMERCURIC NITRATE SUBCUTANEOUSLY IN RABBITS

RABBIT IV (CONTROL)

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	1·5	1·7	1·7	1·75
Hæmoglobin, per cent.	67	80	70	70
Red cells /cu.mm	5,800,000	6,000,000	5,400,000	5,400,000
Colour index	0·6	0·67	0·65	0·65
White cells /cu.mm	6,800	5,400	9,400	9,400
Lymphocytes, per cent.	70	55	70	68
Granulocytes, per cent.	30	45	30	32

RABBIT V

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	1·5	1·65	1·65	1·65
Hæmoglobin, per cent.	80	75	80	74
Red cells /cu.mm	6,100,000	5,900,000	5,900,000	5,600,000
Colour index	0·65	0·63	0·68	0·66
White cells /cu.mm	7,000	8,400	7,000	7,000
Lymphocytes, per cent.	55	65	70	65
Granulocytes, per cent.	45	35	30	35

RABBIT VI

	1 week	2 weeks	3 weeks	4 weeks
Weight (kg.)	1·45	1·6	1·6	1·65
Hæmoglobin, per cent.	71	73	75	75
Red cells /cu.mm	5,550,000	5,200,000	6,200,000	5,750,000
Colour index	0·64	0·7	0·6	0·65
White cells /cu.mm	9,800	5,800	6,200	4,400
Lymphocytes, per cent.	70	70	66	68
Granulocytes, per cent.	30	30	34	32

TABLE III

THE EFFECT OF INTRACISTERNAL INJECTIONS ON THE PRESSURE AND CELL CONTENT OF THE CEREBROSPINAL FLUID

Drug	Concentration per cent.	Weight of dog (kg.)	Pressure of cerebrospinal fluid		White cells per c. mm. after 6 hours
			Initial	After 6 hours	
Chlorocresol ...	0·2	7·85	90	130	4,000*
	0·4	6·0	75	135	4,800*
	0·4	11·0	100	260	10,500*
Phenylmercuric nitrate	0·002	4·3	70	130	3,600*
	0·004	7·7	80	220	2,000*

* All polymorphonuclear.

DISCUSSION

The chronic toxicity of concentrations higher than those recommended in the British Pharmacopœia has been studied in rabbits. There were no abnormalities in the urine, body weight, red and white cell counts or general health of the animals. There was no sign of necrosis at the site of injection.

Therefore, if the present concentrations are not adequate as claimed by Davis and Davison[1], it may be possible to recommend more appropriate concentrations.

Although, according to the British Pharmacopœia instructions, the method of sterilisation by heating with a bactericide is not to be used for the sterilisation of intrathecal and intracisternal injections, this recommendation is not based on any well-known experimental data.

The few experiments done on dogs demonstrate that the introduction into the subarachnoid space of chlorocresol or phenylmercuric nitrate excites powerful cell reactions and causes a rise in the pressure of cerebrospinal fluid, thus justifying on the grounds of actual experiments on animals, the Pharmacopœial instructions prohibiting the use of the method of sterilisation by heating with a bactericide for the sterilisation of intrathecal and intracisternal injections.

SUMMARY

1. Daily injections for 4 weeks of 5 ml. of 0·4 per cent. solution of *p*-chloro-*m*-cresol were given to two rabbits. There were no abnormalities in the urine, body weight, red and white cell contents or general health of the animals.

2. Rabbits receiving daily injections (5 ml.) of 0·004 per cent. solution of phenylmercuric nitrate showed no abnormalities.

3. Pathological examination of kidney, liver and skin at the site of injection showed no degenerative changes or other abnormalities with either bactericide.

4. Intracisternal injections of solutions of *p*-chloro-*m*-cresol and phenylmercuric nitrate produce striking cell reactions in the cerebrospinal fluid and raise its pressure.

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REFERENCES

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3. Wien, *ibid,* 1939, **12,** 212.
4. Bedford, *J. Physiol.*, 1946, **104,** 299.
5. Bedford, *Brit. J. Pharmacol.*, 1948, **3,** 80.