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PROGRESS REPORT

by

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The effect of Diapulse treatment was investigated in mice and rats.

Preliminary experiments were performed on 2460 mice (17-20 gm.) infected with various strains and dilutions of live bacteria given subcutaneously or intraperitoneally. Klebsiella pneumoniae, streptococcus hemolyticus, staphylococcus aureus, pasteurella pseudotuberculosis, escherichia coli, micrococcus tetragenus and salmonella typhimurium were used as infective agents. Mortality in control and Diapulse treated animals was compared and analysed statistically. Differences between treated and control groups depended upon experimental factors such as bacterial strain, way of inoculation, dilution and volume injected (i.e., viable units) as well as treatment intensity (i.e., dial setting). Among the strains tested, Klebsiella and streptococcus appeared most suitable for experimentation. Doses producing a mortality of about 50% over a period of 10 days when injected subcutaneously were preferred.

The mortality related to various bacterial dilutions and volumes as well as to dial settings was tabulated and analysed statistically.

RESULTS:

On Table 1, it may be seen that Diapulse treatment diminished significantly the mortality caused by Klebsiella pneumoniae given subcutaneously 0.1 or 0.2 ml. (dilution 10^{-7}). The protection was greater

in animals treated twice daily with increasing intensities than in animals treated once a day with an invariable 80 x 3 dial setting. When the volume of the infective inoculum was increased to 0.3 or 0.5 ml., the protective effect of Diapulse treatment was reversed and the mortality enhanced. In other words, in severe experimental infections the treatment seemed to act as an additional stress rather than as a means of protection. Such a reaction is conceivable for a treatment such as Diapulse, presumed to stimulate the defense of the host, without influencing the infective agent. When the infection is mild and the defense system has available reserves, the stimulation may increase the resistance of the animal and speed its recovery. Conversely, when the defense system is blocked or annihilated by an overwhelming infection, defense stimulation may fail and the stimulus may act as additional stress. This was apparently the case in mice infected with Klebsiella; an infection with 5 live bacteria (i.e., an 0.1 ml. inoculum) inducing a mortality of only 55%, was favorably influenced by Diapulse treatment and the mortality was reduced. Severe infections with 15 or 25 live bacterial units (i.e., 0.3 or 0.5 ml. inoculum) producing a mortality of 78 or 88% were not influenced by the treatment or were slightly enhanced. (Table 1).

Experiments on larger animals are expected to provide a wider spectrum of experimental infections and treatment modalities.

Table 2 shows the effect of the Diapulse treatment on mice infected with various bacterial strains given subcutaneously. As it may be seen

from the mortality, no protection was conferred in the treated as compared to control groups. However, in one experiment on 120 mice infected with streptococcus hemolyticus (injected subcutaneously 0.2 ml. of a 10^{-6} dilution) the treated group had a significantly lower mortality than the control one. Since the repetition of the experiment did not reproduce the protective effect in the Diapulse treated group, all experiments were pooled and the results on 584 mice presented in Table 2.

Table 3A shows the results of an acute experiment with staphylococcus aureus injected intraperitoneally together with an adjuvant. There is no significant protection in treated as compared to the control group. All control animals were dead within 24 hours following the inoculation. (The Diapulse treatment was performed previous and immediately following the inoculum.)

Experiments on Rats: A pilot experiment performed on prepuber white rats of 50 gm. body weight, showed that systematic exposure to Diapulse waves increases significantly the white blood count in treated as compared to control animals. The average count was 25,400 WBC in treated as compared to 13,500 in control. Differential counts showed an increase in all elements of the white series with an important raise in the proportion of mono and of polymorphonuclear cells (Table 3). The same experiment performed on adult white rats of 150 gm. body weight did not show higher WBC counts in treated as compared to control groups. Red blood cells and hemoglobin were apparently unaffected by the treatment in these adult rats (Table 4).

CONCLUSIONS:

The one pertinent conclusion of the aforegoing study is the need for further and deeper investigation. This conclusion is justified by the following: 1) The significant results obtained in mice infected with Klebsiella could not be reproduced with other bacterial strains. 2) The impressive effect of the Diapulse treatment upon the WBC series in prepuber rats could not be obtained in older animals. 3) Changes in the dial setting of the machine reversed the effect of the treatment and so did a change in the volume of the inoculum. 4) Animal species, size, age and sex, bacterial strain, dilution and volume, way of inoculation, intensity of treatment, time, frequency and dial setting, all were factors as important as to be able to reverse the effect of the treatment from desirable to undesirable and vice versa. 5) The accurate measurement of the machine output still remains of utmost importance in small sized animals. Due to unsuspected failure of the machines, experiments on many hundreds of animals had to be discarded. We still need a more reliable instrument to detect machine failure especially on low settings.

Before restarting the experiments on diseased animals a systematic study of the normal physiologic reaction to the treatment of various animal species is necessary in order to separate the general from the particular and the useful from the harmful. This may provide positive indications for the best and most effective use of the machines. Larger animals (rabbits, dogs and possibly monkeys) could be used for a larger

variety of dial settings.

It is for such reasons that we may have to deviate from the initially submitted plan in order to speed the progress of the investigation.

TABLE 1

EFFECT OF DIAPULSE TREATMENT UPON MORTALITY OF MICE INOCULATED SUBCUTANEOUSLY WITH VARIOUS VOLUMES OF A 10^{-7} DILUTION OF KLEBSIELLA PNEUMONIAE

INOCULUM	TOTAL NO. OF ANIMALS	MORTALITY IN CONTROL	MORTALITY IN TREATED 80 x 3	MORTALITY IN TREATED Variable*
0.1 cc	221	56%	25%	13%
0.2 cc	180	39%	58%	28%
0.3 cc	80	78%	90%	-----
0.5 cc	80	88%	95%	-----

* In the variable treatment the animals were treated b.i.d. with progressively increasing-decreasing dial settings between 80 x 3 and 300 x 3.

TABLE 2

EFFECT OF DIAPULSE TREATMENT UPON MORTALITY OF MICE INFECTED WITH VARIOUS BACTERIAL STRAINS SUBCUTANEOUSLY

STRAIN	TOTAL NO. ANIMALS	MORTALITY	
		CONTROL	DIAPULSE TREATED
Streptococcus	584	61%	58%
Salmonella	20	50%	90%
Pasteurella	20	30%	50%
Escherichia	60	3%	3%
Micrococcus	40	10%	5%

TABLE 3

AVERAGE EFFECT OF DIAPULSE TREATMENT UPON WHITE BLOOD COUNT IN PREPUBER WHITE RATS (50 gm. body weight)

GROUP	NO. OF ANIMALS	WBC	POLY	LYMPH	MONO	EOS
Control	7	13,480	2,676	10,379	326	76
Treated	7	25,390	6,964	16,429	1,812	180

TABLE 3A

EFFECT OF DIAPULSE TREATMENT UPON MORTALITY OF MICE INFECTED INTRAPERITONEALLY WITH STAPHYLOCOCCUS AUREUS

GROUP	NO. ANIMALS	MORTALITY %
Control	40	100
Treated	40	95

TABLE 4

AVERAGE EFFECT OF DIAPULSE TREATMENT UPON BLOOD COUNT IN ADULT WHITE RAT (150 gm. body weight)

TREATMENT	NO. ANIMALS	HEMATOCRIT %	RBC	HGB. gm.%	WBC	POLY	LYMPH	MONO
Control	5	27.6	3,530,000	12.10	13235	2527	10006	596
80 x 3	5	38.5	3,950,000	12.80	8025	1477	6139	409
300 x 1	5	38	4,114,000	13.2	9570	2459	6938	258
300 x 3	5	37.4	4,000,000	13.12	11475	2811	8732	379