

RESULTS OF INDEPENDANT INVESTIGATIONS ON RS-I SOLUTION

EXP.1: Bioassay of LEUKOTRIENES from Sheep intestine.

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LOCATION: Ministry of Agriculture & Fisheries, Wallaceville, Wellington, NZ.

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ACCOUNT:

Bioassays using smooth muscle preparations have contributed substantially to advances in 'eicosanoid' research, providing a sensitive means of detecting and quantifying spasmogenic compounds (S-E, Dahlen et al.,1987).

Such bioassay techniques have been utilised by the present investigators to examine the effector mediators involved in the resistance of sheep to gastrointestinal nematode parasites.

In these experiments, electrically stimulated guinea pig ileum or longitudinal muscle strips were perfused in a Res-Del[®] horizontal, physiological organ bath with RS-I mammalian saline (Rees,1985). Intestinal mucus from nematode-resistant sheep was shown to contain leukotrienes. However, such mucus did not give positive radioimmunoassay (RIA) for leukotriene-C4 unless greatly diluted (thus indicating that the RIA-binding site was not accessible to the antibody) and therein demonstrates the usefulness of a bioassay procedure.

The use of RS-I saline, in comparison to that of Tyrode's or Hank's physiological salines, indicated that the contractile activity of isolated guinea pig-ileal tissue was far stronger and gave more consistent results in RS-I saline (see Figs. 1,2 & 3). In addition, it was found that in RS-I saline, guinea-pig ileal biopsies could be maintained for at least 7 days at 0-4 °C and still be responsive to leukotrienes (see Fig.3).

Interestingly, RS-I mammalian saline has been successfully used for the *in vitro* maintenance of sheep ileal tissue (see Fig. 4) and also for the preservation of purified populations of globule leukocyte cells from sheep intestinal mucosa.

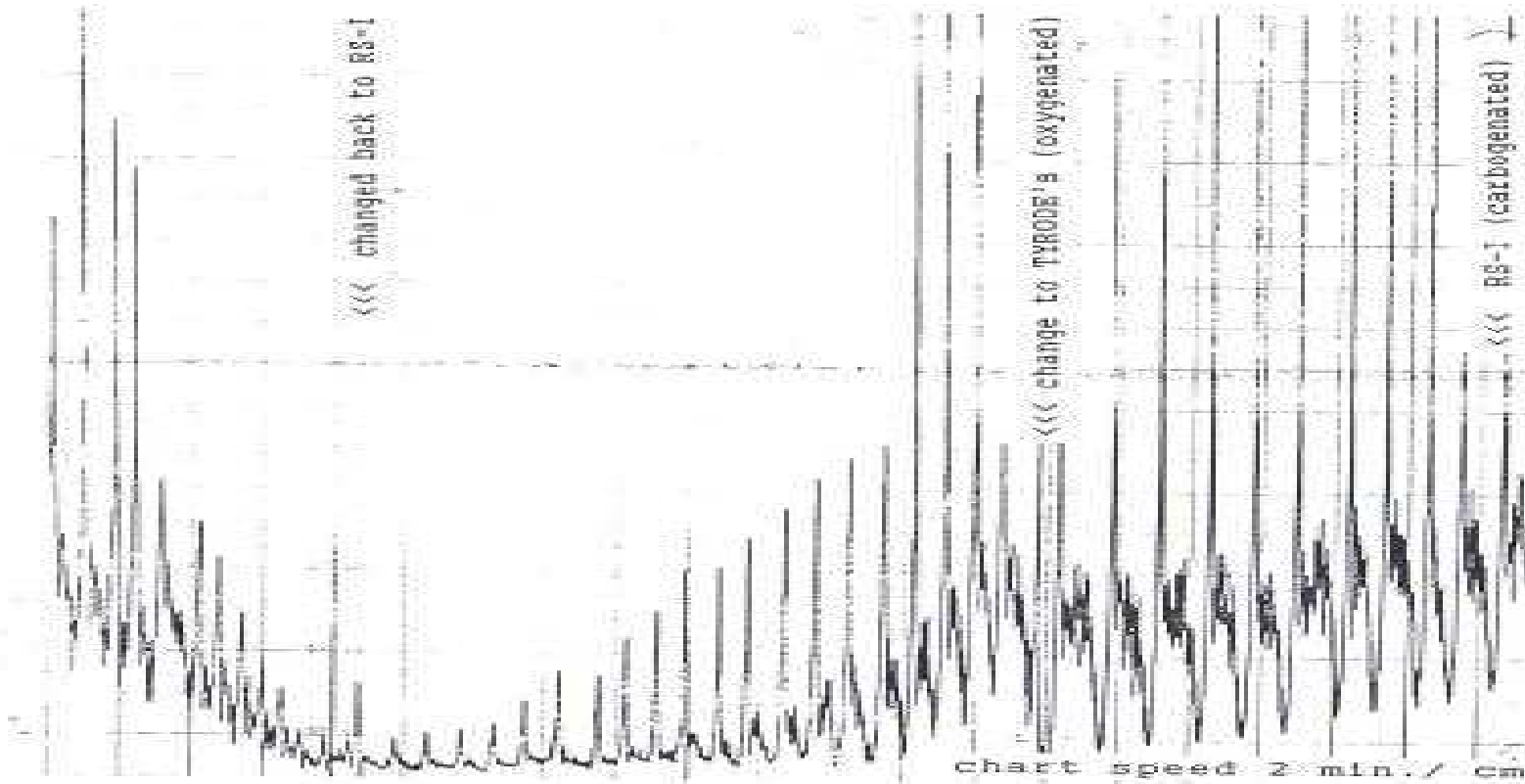


Fig. 1 Effect of alternate perfusions of TYRODE AND RS-I oxygenated salines at 37°C on electrically induced contractions of isolated, guinea—pig longitudinal ileal muscle. Note the **90%** decrease in activity over a 15 min. perfusion period in TYRODE's saline and the **100%** recovery following 9 min. perfusion with RS-I mammalian saline.

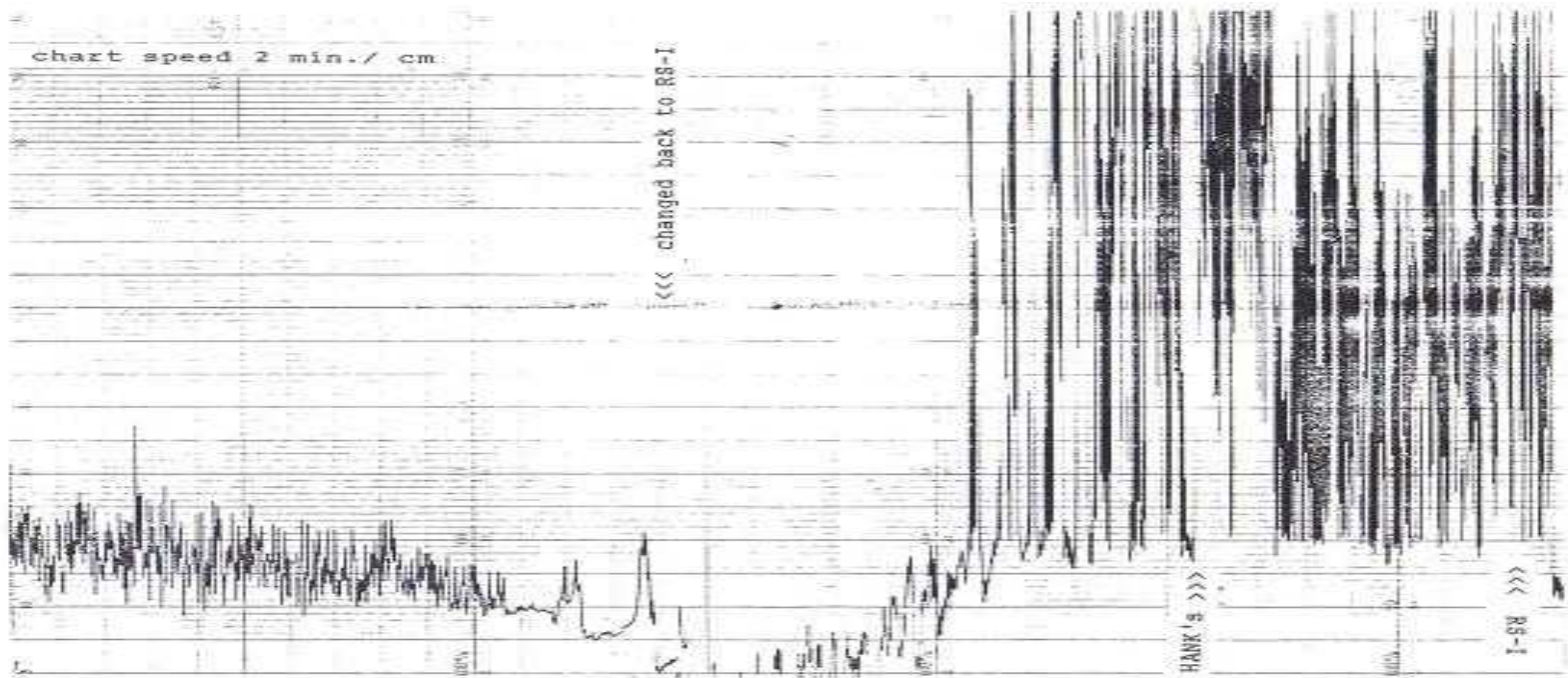


Fig. 2 Effect of alternate perfusions of HANKS and RS-I oxygenated salines at 37°C on electrically induced contractions of isolated, guinea-pig longitudinal ileal muscle. Note the immediate decrease in contractile activity following perfusion with HANK'S phosphate buffered saline and its irreversibility after 15 min. perfusion with RS-I saline.

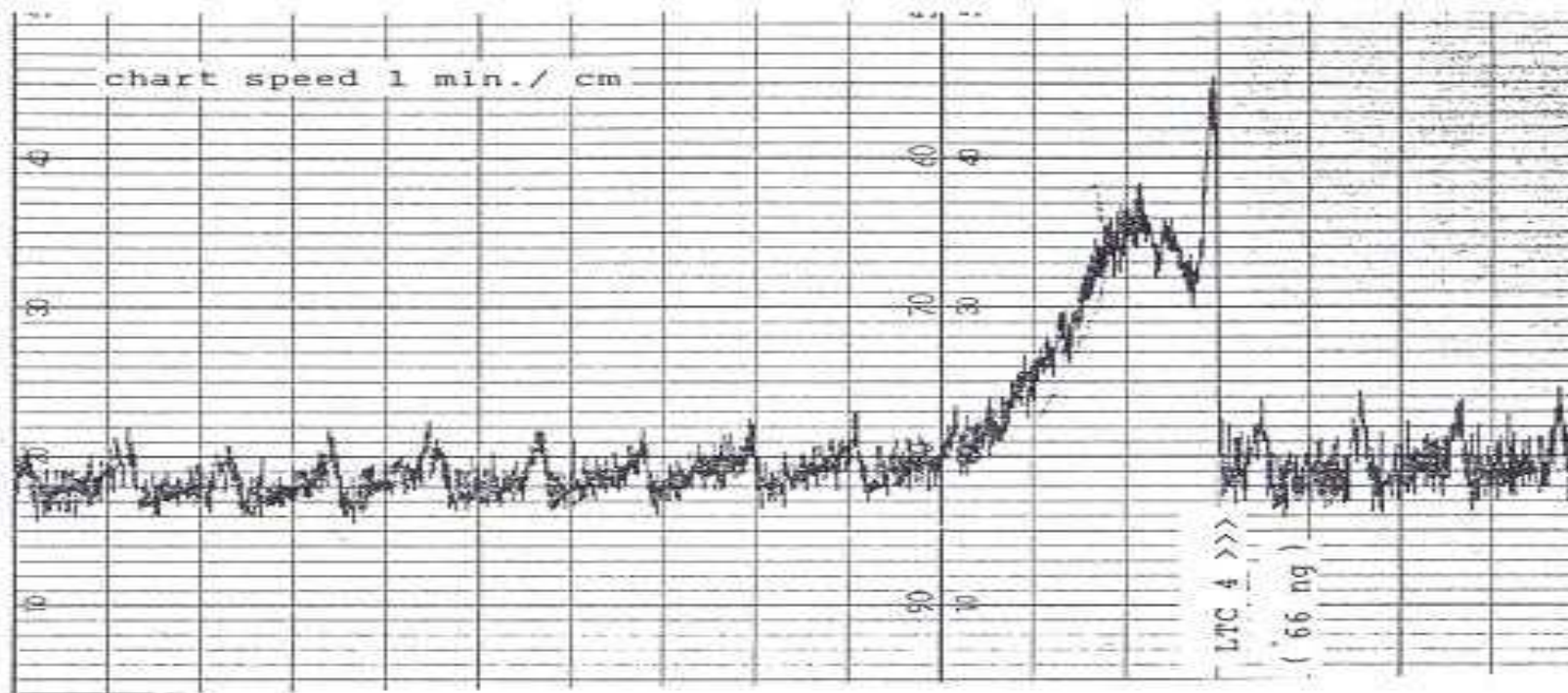


Fig.3 The effect of topically applied leukotriene-C4 on the spontaneous contractile activity of guinea-pig ileal longitudinal muscle strips maintained *in vitro* in RS-I mammalian saline S 37°C. (** This preparation had been stored in RS-I saline at 0-4° C for 7 days prior to this bioassay test).

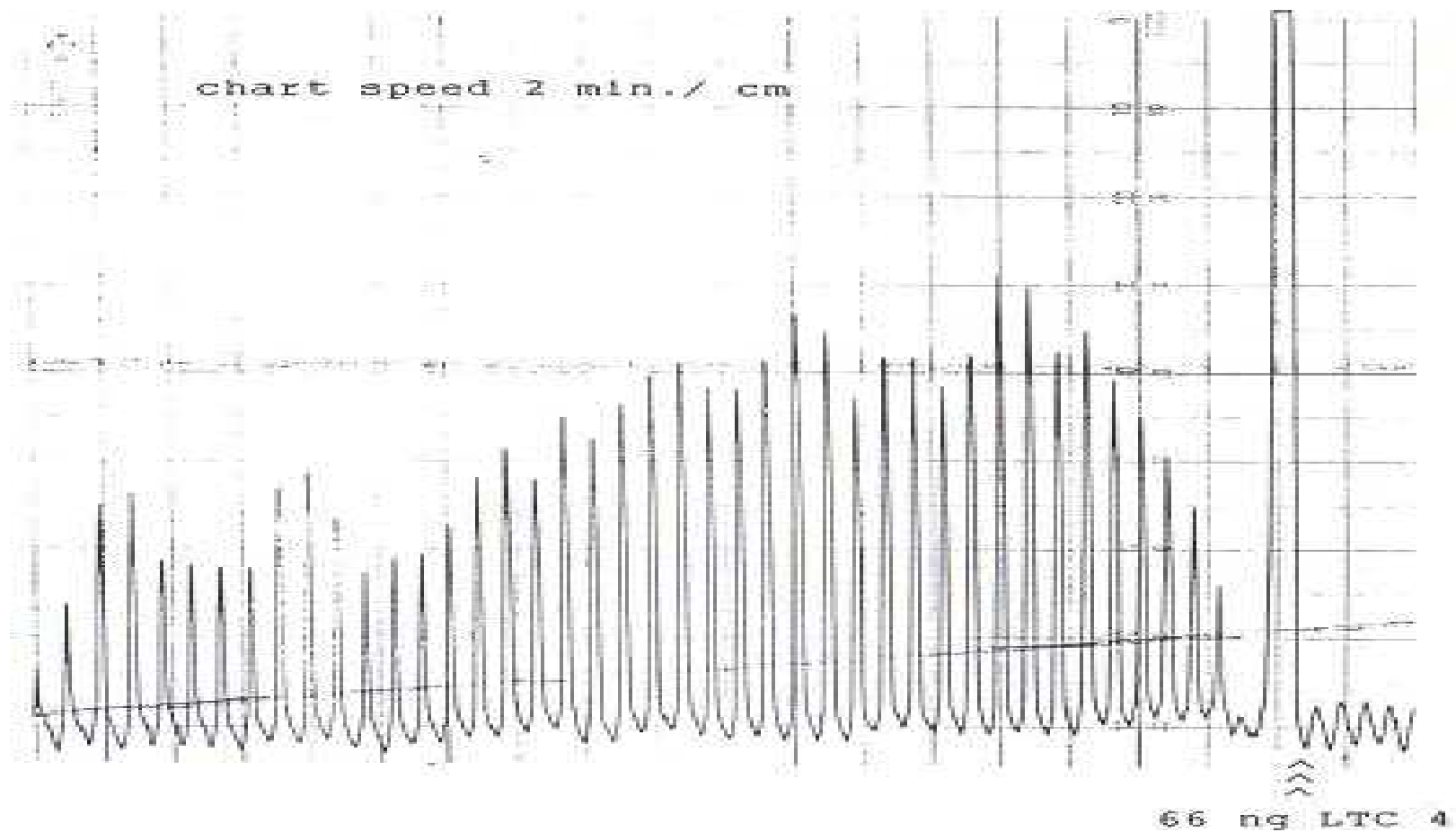


Fig. 4. The effect of topically applied leukotriene-C4 on isolated ileal biopsies from the sheep perfused with oxygenated RS-I saline (incl. insulin) at 37 °C . Note the dramatic increase in the amplitude of the electrically evoked contractions following the initial, large LTC4-induced contracture.

Audit Trial 3 Comparative study on AQIX® RS-I solution as a perfusion and preservation media for nematode-infected sheep intestine and leucotrienne bioassay against the guinea pig ileum preparation.

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	Function	Leucotriene Production	Functional Period	Bioassay Response
Tyrodes [6]	< 1 hr	variable	1 - 2 hr	< 1 hr @ 1000 µg/cm ³
Hanks [5]	< 15 min	negligible	< 25 min	NONE @1000 µg/cm ³
AQIX® RS-I [12]	1 - 7 days	optimal	1 - 7 days	Constant @ 60 µg/cm ³