CUTTER LABORATORIES

Biochemical Research Department
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DISTRIBUTION

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III. Australia Antigen/Serum Hepatitis (Au/SH)

A. Inactivation or Removal from Plasma Products

In an attempt to inactivate HAA which might be present in Konyne, a bulk desiccated powder of PTC FR 41301B was dissolved in 0.05M Na-citrate-0.088M NaCl at various concentrations and heated at 60°C for 10 hours. PTC solutions at 12.5% and 25% levels completely gelled within a few hours. None of the additives tested (0.5% albumin, 0.3M glycine, 0.02M Na-caprylate and 0.02M acetyltryptophan, and 50% glycerol) prevented gelation. At 4% and 8% levels with various additives, all but 8% with 0.02M Na-caprylate and 0.02M acetyltryptophan escaped gelation, but factor IX activity was completely destroyed by this heat treatment. Therefore, the pasteurization of PTC solution does not appear feasible.

As a next experiment PTC was pasteurized in dry form. Both bulk desiccated powder (no salt) and final desiccated plug (salts added) were held at 60°C for 10 and 20 hours, and assayed after reconstitution. As shown in Table XII the loss of in vitro factor IX activity was considerable in the absence of salts, although very little destruction took place in the presence of salts.

Table XII. Heat treatment of PTC in dry form in the absence of salts.

Untreated control	43.4 u (38.5, 44.1, 47.6)
10 hours at 60°C	32.3 u (35.0, 32.6, 29.6)
20 hours at 60°C	28.1 u (23.8, 28.4, 32.2)

To test the effect of this heat treatment upon virus, PTC solution (FR 41301B dissolved in 0.05M Na-citrate-0.088M NaCl) was seeded with blue tongue virus (BTV) and infectious bovine rheinotracheitis virus (IBR), lyophilized, and heated at 60°C for 20 hours. However, this treatment was found to have no inactivating effect upon these viruses. Therefore, it appears infeasible to inactivate HAA by this treatment.

β-Propiolactone (BPL) was tested at various levels for its effect. Results are summarized in Table XIII.

Table XIII. Treatment of PTC with BPL

BPL treatment	% Loss of PTC
0.2%, 4°C, 17 hours	90%
0.3%, 4°C, 17 hours	> 90%
0.4%, 4°C, 17 hours	> 90%
0.1%, 4°C, overnight	84%
0.2%, 4°C, overnight	95%
0.05%, room temp., overnight	71%
0.1%, room temp., overnight	83%

Benzalkonium chloride was used in one experiment at 0.1% for 30 minutes at room temperature. The treated PTC solution was dialyzed, lyophilized and assayed. By this treatment the loss of PTC activity was 28%. According to literature (Horst, et al.) the minimum level of benzalkonium chloride required for inactivating HAA is 1%. Accordingly, it appears impossible to inactivate HAA without destroying PTC.

Ethylene oxide was tested both in gaseous and liquid form. For the former, a commercial mixture of 12% ethylene oxide and 88% inert gas was used. In Exp. 1360-89, a PTC solution prepared from a bulk powder FR 41301B was treated with pure ethylene oxide in liquid form at 0.1%, 0.2%, and 0.5% levels at 4°C for periods up to 12 hours. Samples removed at various time intervals were lyophilized and assayed for PTC activity. Results are shown in Table XIV.

Table XIV. PTC treated with liquid ethylene oxide at low levels.

Treatment	2 hrs.	4 hrs.	6 hrs.	12 hrs.
0.1% EtO	32.2 u	27.3 u	31.2 u	28.0 u
0.2% EtO	30.5 u	28.7 u	26.6 u	29.8 u
0.5% EtO	35.7u(?)	32.2u	29.4u	29.8u
Untreated control		28.7u		

Assay results did not show expected loss of activity with time and concentration. Treatment with EtO at these low levels at 4°C does not appear to destroy PTC activity. However, it is highly questionable whether HAA is inactivated at these levels. In Exp. 1360-90 (5/24), liquid ethylene oxide was used at 2% and 5% levels for 20 hours at room temperature and 4°C, respectively. Results are shown in Table XV.

Table XV. PTC treated with liquid ethylene oxide at high levels.

Treatment	PTC	
2% EtO at room temp. for 20 hrs.	~ 4.8u	*
5% EtO at 4°C for 20 hrs.	~ 13.7u	*
Untreated control kept at 4°C for 20 hrs.	25.7u	

* These values were obtained by extrapolation on standard curve.

At these high levels the destruction of PTC activity was marked. In both Exp. 1360-89 and 1360-90 (5/24), liquid ethylene oxide was added by using a chilled capillary pipet. However, due to low boiling point the exact measurement of liquid ethylene oxide was extremely difficult and the amount actually added to PTC was nothing more than an approximate volume.

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In Exp. 1360-90 (5/23) and 1360-91, a bulk desiccated powder (#43326A) prepared by desiccating 250 ml. eluate in a small tray was treated with ethylene oxide gas at 36°C and 10°C, respectively, for 5 hours. PTC thus treated was dissolved in 0.05M Na-citrate-0.088M NaCl in the usual manner and assayed. Results are shown in Table XVI.

Table XVI. PTC treated with ethylene oxide gas

Treatment	% Destruction of PTC
36°C for 5 hours	78.5%
10°C for 5 hours	15%

It is shown that temperature has a pronounced effect upon the effect of ethylene oxide.

In order to test the viricidal effect of ethylene oxide as used in the present study, PTC powder (#41301B) was dissolved in 0.01M Na-citrate (2.4 gm/60 ml.), and adjusted to pH 7.5 with 1N NaOH. Twenty milliliters of the PTC solution were poured into a 95 mm Petri dish, seeded with IBR, lyophilized, and exposed to ethylene oxide gas in a glass desiccator at room temperature for 5 hours. By this treatment there was 100% inactivation of seeded virus and PTC activity. Search for an optimum condition for inactivating HAA with a minimum loss of PTC activity is in progress.

B. Migration During Preparation of Clotting Factors

Near the end of this quarter, we prepared AHF, PTC and fibrinogen from HAA-positive plasma. In process samples were collected and are being assayed for HAA by both AGD and CEP tests. Arrangements have been made to assay the samples by radioimmune methods also. The data will be collected and reported in the next quarterly.

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