

**The efficacy of fluid and freeze dried
antitoxic sera during the Lifetime**

by

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Ever since 1940 Razi Institute has been producing refined anti-sera against various diseases. The Institute has particularly been interested to prepare these anti-sera with as high potency as possible. In order to insure a potent serum until the date of its expiration as indicated on the labels the sera have been usually made 20% more potent at the time of their distribution. We have stored refined anti-sera at different temperature and have studied the effect of storage on the potency of the anti-sera. It seems that various conditions as the change in temperature would effect the physical appearance of these anti-sera, as well as their potency when the refined and concentrate anti-sera were stored at room temperature, particularly during the summer season would cause the sera to be clotted and consequently lose of their usefulness.

Lyophilisation of sera— During the World War II an extensive work was done in order to substitute dried serum and plasma for the whole blood (2). The process of drying was later on applied to the other biological products.

Lyophilisation has proved to be valuable for certain therapeutic antisera. In our knowledge Anti-snake venom serum was the first therapeutic material which was largely lyophilised. Hazra et all (3) claim that "the lyophilised Anti-snake venom serum may be stored in any cool dark place and may even be carried in a havesack on one's back if an occasion demands it. Even stored at room temperature, it is expected, it will keep its full potency for ten years or so".

At the present time in the U.S.A. and in some European countries the anti-venom serum as well as a lot of other therapeutic refined sera are used in lyophilised form.

Is it interesting to note that if these globulins could be lyophilised without losing their activity, their reconstitution is rather slow. Moreover, in case of lyophilised crude or salted out sera on reconstitution the solution is opalescent. This insolubility may be due to the presence of lipids not extracted before freeze drying.

For a long time, in case of enzyme digest, refined Diphtheria or Tetanus antitoxins we have observed in collaboration with H. Mirchamsy and H. Manhoury (unpublished data) that the reconstitution of the lyophilised globulins gives a solution free from opalescence.

It was found that the technic of delipidation of More and Rao (5) or that of the Haffkine Institute for removing of lipids will bring some denaturation in the product.

We believe the removal of lipids by ether and toluol during the purification with Aluminium hydroxide will eliminate the excess of lipids and there is no need for further treatment of such globulins before freeze drying.

The lyophilisation is carried out by freezing the globulin solution and drying under vacuum in Edward's apparatus. The final moisture of the product is less than 1%.

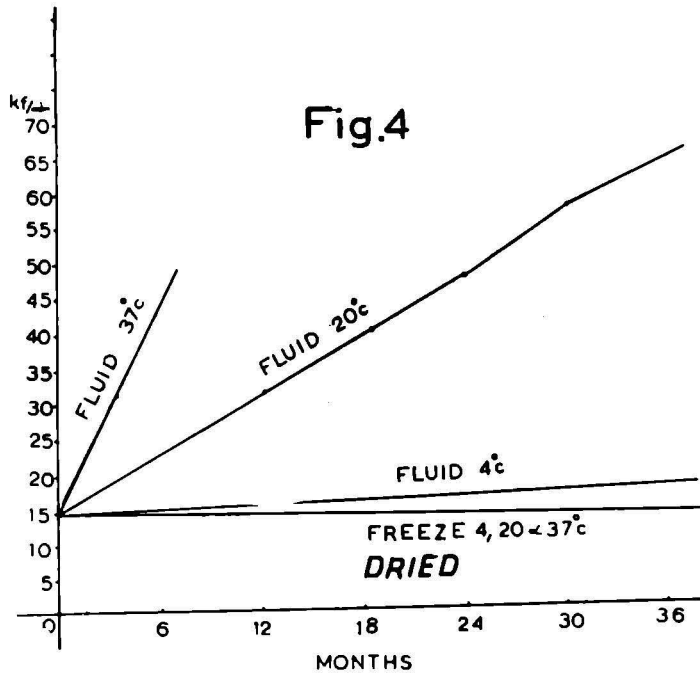
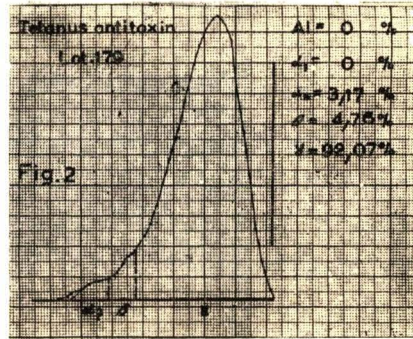
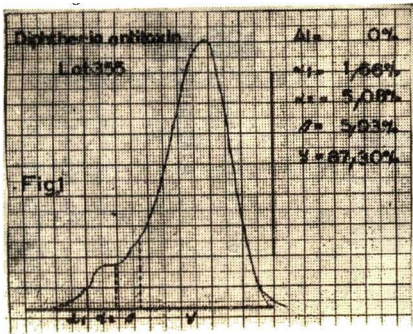
Following is a representation of some of our studies in regard to the effect of temperature upon anti-sera as has been studied in this laboratory.

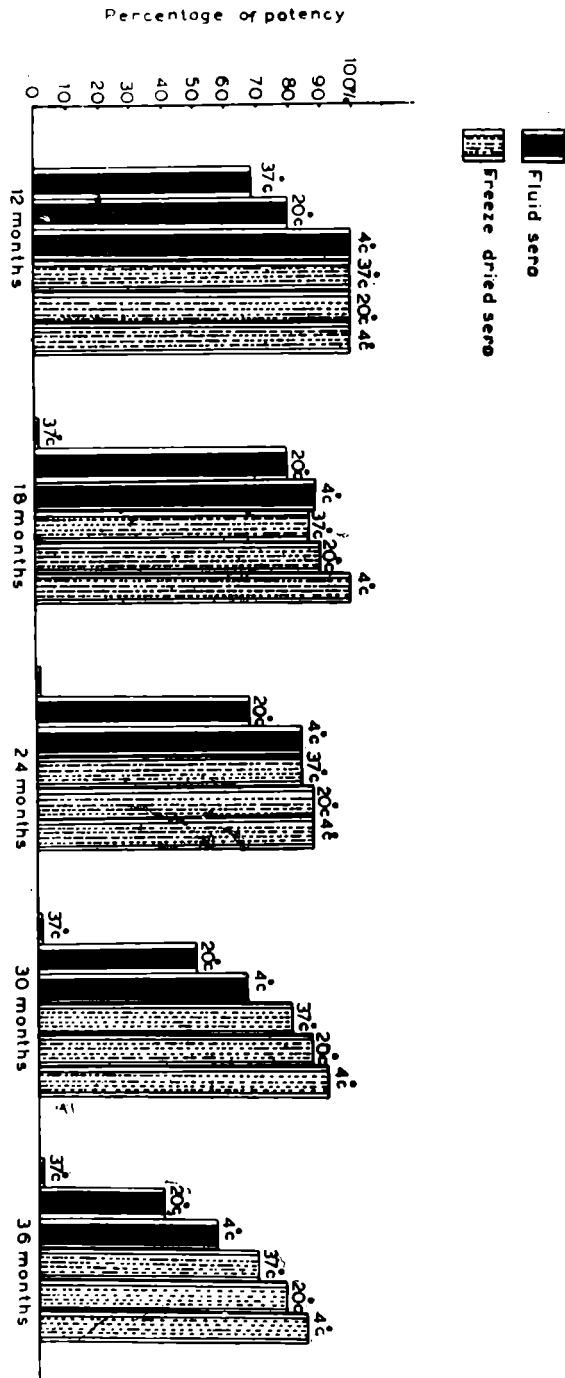
Experimental:

a) **Diphtheria Antitoxin** - 8000 ml of the enzyme digest refined Diphtheria Antitoxin lot No 355 (fig.1). Prepared as described Elsewhere (1) was partly distributed in vials of 5 ml, freeze dried and divided in 3 groups of 200 vials and stored at 4C, 20C and 37C. Another part was distributed aseptically in vials of 10 ml and kept under the same temperature.

b) **Tetanus Antitoxin** - 8000 ml of the enzyme treated refined Tetanus Antitoxin lot No 179 (fig.2) was distributed in the same way as the above mentioned Diphtheria Antitoxin. 10 vials of each group were taken at random immediately after distribution and lyophilisation.

In case of Diphtheria, the potency of the pooled samples was verified by ramon's flocculation test (6), and by modified Römer intracutaneous injection. For tetanus antitoxin titration, both flocculation and Ipsen's





(4) mice test were used. The experiment is carried out in the same way 12 to 36 months after keeping the samples at the temperature indicated above.

Effect of temperature on physical condition of Anti-sera

The sera that were freeze dried and also the sera that were kept at 4°C as indicated above did show any change in their physical appearance. The fluid samples that were kept at 20°C and 37°C however, showed a change in color. These sera would turn gradually red and then into dark and brown and would eventually clot.

Potency - There is undoubtedly some decrease in potency of sera stored for a long time. This change is negligible in case of freeze dried sera whereas the decrease in titer for the fluid sera was much more severe. By keeping sera at 37°C as it is shown in fig. 3, the potency diminished after the period of 3 years at a rate of 60% of the initial titre but the same product lyophilised and stored at 37°C kept 85 to 90 percent of its potency after 3 years.

There was at the same time an increase in Kf (time of flocculation) but this change was only observed in heated fluid sera, the Kf for freeze dried or fluid sera stored at low temperature remained unchanged (Fig. 4). The avidity of the heated sera was more or less affected during the period of conservation. It was however, stabilised for dried sera as well as for cold keeping products.

Another fact is that the avidity and Kf are less affected in case of Tetanus Antitoxin comparing with Diphtheria Antitoxin.

We believe these findings have significant value in tropical countries where the correct conservation of the biological products are sometimes neglected.

Summary

There is a marked drop in potency and avidity of the refined anti-toxic sera, when they are kept in fluid state at room temperature. On the other hand, there is not any clear change in the fluid products stored at low temperature. In case of freeze-dried sera, no change was noticed even after keeping them for almost three years at 37°C

References

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