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# CONCENTRATION OF ANTIRABIES SERUM

by

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All workers who have so far published methods for the concentration of antirabies serum use procedures based on fractionation: Habel (1) employs sodium sulfate, Koprowski et al. (2) and Devi et al. (3) employ ammonium sulfate.

We have tried to apply for this purpose the enzymatic digestion procedures which we utilized for the concentration and purification of antitoxic sera. In parallel with the ammonium sulfate concentration method (modified Wadsworth technique, Standard Methods, 1947), which we normally employ, we have tried out two enzymatic digestion methods: firstly, that of Pope formula due to the Michigan Department of Health, variant developed at the Razi Institute, (4) and, secondly, that of Wang & Lin. (5)

The attached table (Table 1) shows the results obtained with these three methods: the enzymatic digestion procedures give products of the same order as regards concentration but with a very much lower yield

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★ WHO/Rabies/130,27 November 1959

1 Habel, K. (1945) **Publ. Hlth Rep. (Wash.) 60,** 545

2 Koprowski, H., Van der Scheer, J. & Black J. (1950) **Amer. J. Med. 8,** 412

3 Devl, P., D'Silva, C. B. & Ahuja, M. L. (1956) **Indian J. med. Res. 44,** 157

4 Delsal, J. L. & Mirchamsy, H. (1953) **Rev. Immun. 17,** 110

5 Wang, S. P. & Lin, C. C. (1957) **Formosan Med. Assoc. 56,** 10.

than that given by the ammonium sulfate fractionation method (5.6 to 22% instead of 40 to 62%).

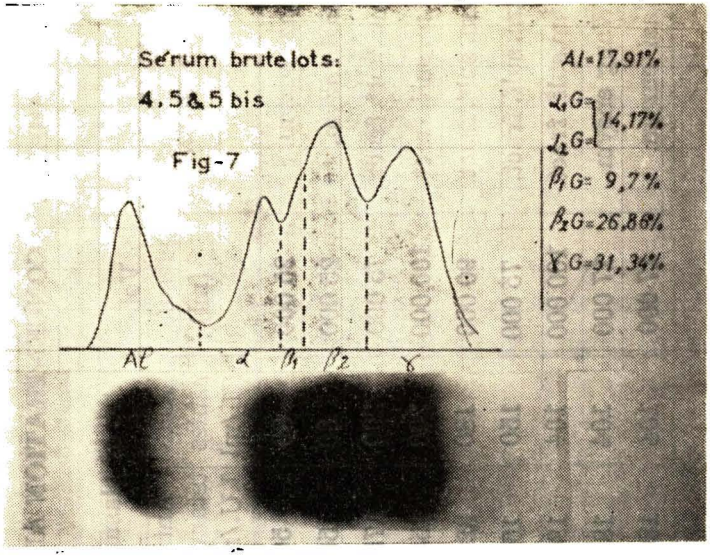
Under our working conditions, the ammonium sulfate concentration method with decolorization by means of aluminium hydroxide (Wadsworth method) gives a product with a 3 to 6 times higher concentration of neutralizing antibody than the crude serum and seems to be the method of choice.

We are at present studying the use of these very high-titre sera for instillation into wounds left by bites; for intramuscular injection, these sera can be rediluted to the desired titre with a particularly low total protein concentration.

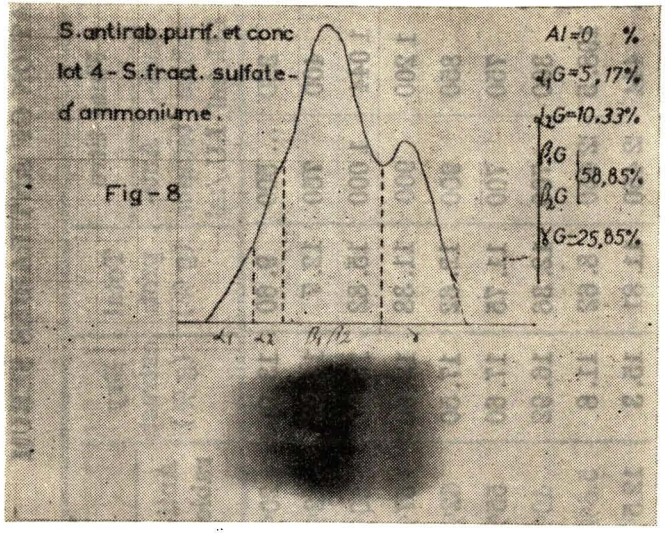
The annexed electrophoretic curves (Figs 1-4) show the composition of the concentrated sera obtained by the different methods.

**TABLE 1. CONCENTRATION AND PURIFICATION OF ANTIRABIES SERUM**

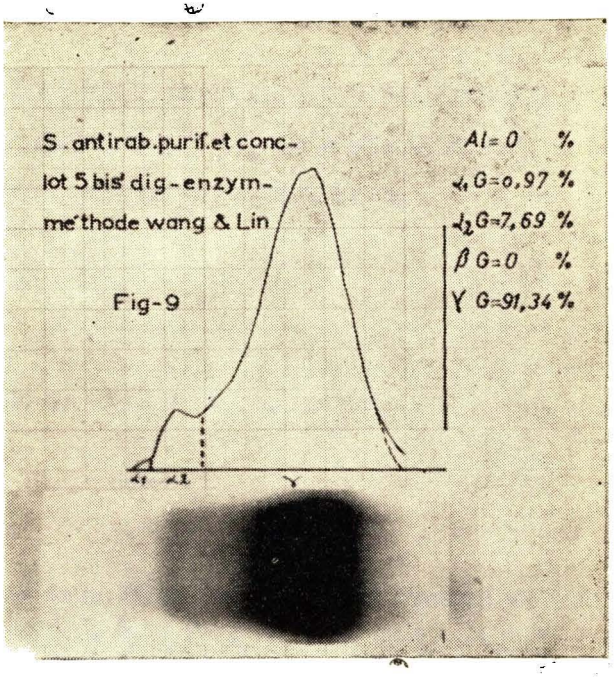
| Lot No. | Method concentre. | Vol. plasma (ml) | Titre of plasma | | Final vol. (ml) | Final titre | | Total prot. (g %) | Dry extract (g %) | Yield % | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Anti-rabies I.U./ml | Anti-tetanus I.U./ml | | Anti-rabies I.U./ml | Anti-tetanus I.U./ml | | | Anti-rabies | Anti-tetanus |
| 1 | Razi enzym. | 25 000 | 80 | 50 | 1 250 | 320 | ... 500 | 9. 60 | 13. 05 | 20% | 50% |
| 2 | Razi enzym. | 25 000 | 80 | 50 | 1 100 | 400 | 750 | 12. 7 | 14. 93 | 22% | 65% |
| 3 | Am. sulf. fract. | 75 000 | 200 | 175 | 7 200 | 1 044 | 1 000 | 15. 82 | 18. 80 | 50% | 54% |
| 4 | Am. sulf. fract. | 100 000 | 200 | 150 | 9 500 | 1 200 | 900 | 11. 38 | 13. 00 | 57% | 57% |
| 5 | Am. sulf. fract. | 90 000 | 180 | 220 | 12 000 | 850 | 900 | 12. 62 | 17. 50 | 62% | 54% |
| 6 | Am. sulf. fract. | 75 000 | 150 | 160 | 8 250 | 750 | 700 | 11. 75 | 17. 60 | 55% | 48% |
| 7 | Am.sulf. fract. | 100 000 | 104 | 180 | 14 000 | 300 | 700 | 12. 36 | 16. 92 | 40% | 54% |
| 8 | Wang enzym. | 17 000 | 104 | 180 | 500 | 198. 5 | 2 450 | 8. 62 | 11. 8 | 5.6% | 40% |
| 9 | Razi enzym. | 17 000 | 104 | 180 | 500 | 444 | 2 450 | 11. 81 | 15. 3 | 12.5% | 40% |



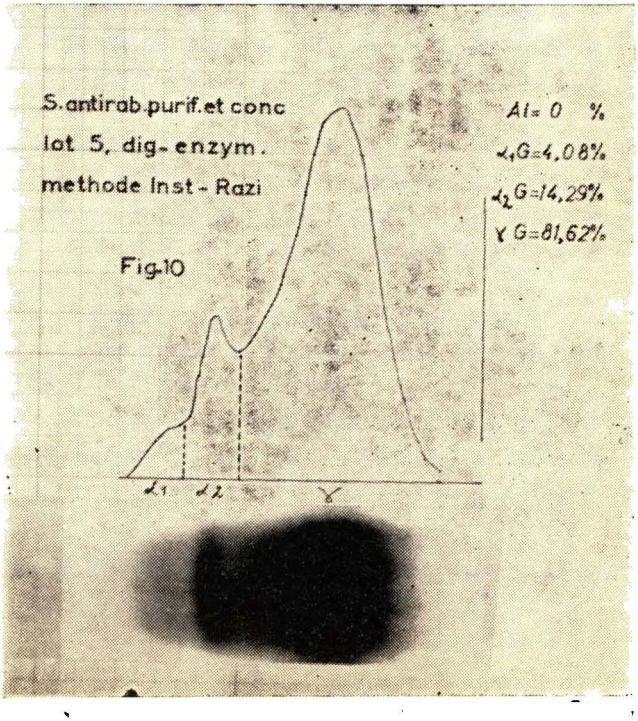

**Crude serum: lots 4, 5 and 5 bis**




**Purif. and conc antirab, s.**
**lot 4-ammon. sulfate s. fract.**




**Purif and conc. antirab. s. lot 5, Razi Inst. enzym. dig. method**




**Purif and conc. antirab. s. lot 5 bis Wang & Lin enzym. dig. method**