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The Relation of Serum Fractions to Serum Sickness in Rabbits.*

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We have previously shown that injection of rabbits with from 5 to 10 cc. of horse serum per kilo of body weight, will cause the appearance of serum sickness in a large percentage of the animals. The reactions which we have considered as those of serum sickness are noted upon the rabbits' ears 4 to 8 days after injection.[1]

An attempt was made to determine whether or not the fractions euglobulin, pseudoglobulin and albumin of horse serum, might be or might contain the agent related to the causation of serum disease. Accordingly, these serum fractions[2] were injected into white rabbits in various dosages and with the results listed in Table I.

TABLE I.

Serum Protein Fraction	Mg. N. Inj. per kilo	No. Rabbits Injected	% Positive
Euglobulin	34-43	19	0
"	69-84	20	0
"	150	30	7
Pseudoglobulin	35	24	0
"	71-76	39	28
"	100	58	41
"	150	25	80
Albumin	43-54	18	0
"	86-108	14	0
"	150	20	0

The horse serum fractions used were from both normal and diphtheria toxin immune animals. In the present and in previous comparative studies, we have noted little or no difference between normal and immune sera, or between their respective fractions, in ability to cause serum sickness.

It is to be noted that a dose (per kilo of body weight) of 150 mg. of euglobulin nitrogen brought about serum sickness in 7% of 30 animals, while smaller doses of euglobulin were without effect in causing serum sickness. Pseudoglobulin was more active, with

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[1] Fleisher, Moyer S., and Jones, Lloyd, *J. Exp. Med.*, 1931, **54**, 597.

[2] These fractions were kindly supplied by Sharpe and Dohme.

reference to the amounts injected, since a dosage of 150 mg. nitrogen per kilo brought about serum sickness in 80% of 25 animals. Smaller doses (*i. e.*, from 71 to 100 mg. nitrogen) of this fraction resulted in a decreased occurrence of serum sickness and since none of the animals receiving 35 mg. of nitrogen per kilo developed the disease, it may be inferred that under these conditions the threshold for development of serum sickness in the most susceptible animals lies at a dosage of between 35 and 71 mg. pseudoglobulin nitrogen per kilo. The albumin fraction, though administered in a dosage as large as 150 mg. nitrogen per kilo was without effect in causing serum sickness.

Under these conditions, it may be assumed that the inciting agent of serum sickness is encountered primarily in the pseudoglobulin fraction of horse serum.

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Preparation of Prolan, Theelin and Theelol from the Same Urine.

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No doubt the possibility of preparing prolan, theelin and theelol from the same urine has occurred to many investigators interested in sex hormones. In this note we have given the adaptation of processes published from this laboratory which permits the preparation of the 3 substances. The combined methods which have been in constant use for about one year give a satisfactory yield of all 3 compounds.

The prolan is made by the benzoic acid process described by Katzman and Doisy.[1] The filtered urine is strongly acidified with hydrochloric acid and after a few days the supernatant liquid siphoned from the precipitate. The urine is extracted with butyl alcohol in the continuous extraction apparatus (Veler, Thayer and Doisy[2]). The solvent is distilled off, the residue leached with benzene and the benzene removed by distillation. The residue from 100 gallons of urine is dissolved in 1500 cc. of 80% ethyl alcohol

[1] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

[2] Veler, C. D., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.