

nitrogen analyzed immediately after removal of the tubes from the press and "48 hrs.," that nitrogen found in aliquots after remaining in a thermostat at 37° for that period.

Sample Number.	Pressure.	Time.	Initial.	48 Hours.
Control (septic).....	xx	xx	0.50 c.c. 0.1 N.	0.80 c.c. 0.1 N.
I. septic.....	75(10) <sup>3</sup>	5 mins.		.90
II. sterile.....	16(10) <sup>4</sup>	2 hrs.		.50
III. sterile.....	(10) <sup>5</sup>	16 hrs.		1.40
IV. sterile.....	(10) <sup>5</sup>	1 min.		.50

The explanation of number III being at variance with number IV, where both are at the same degree of compression, doubtless lies in the fact that during the longer period of exposure (16 hrs.), some enzyme action may proceed before inhibition sets in, especially as the temperature rises somewhat<sup>4</sup> during this prolonged time of exposure.

It is evident that the method can be used only with the greatest care to adjust the pressure so that complete inhibition does not occur, while at the same time, all bacteria<sup>5</sup> are killed, which is of doubtful practicability.

At these pressures, egg albumen is coagulated (B.H.H.), but in the case of the tissue from the rabbit's liver, there is little evidence of change in the colloidal dispersion, although the Ringer's Solution used to make up the liver mass to 20 per cent. suspension separates in the tubes during compression from the semi-solid mass.

### 73 (1533)

#### The relation between the disappearance of foreign proteins from the circulation and the formation of antibodies.

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Some observations which Dr. Rackemann and one of us made a few years ago indicated that when serum disease followed the injection of horse serum in human beings anti-bodies, such as

<sup>4</sup> The cylinder is cooled during the period of compression.

<sup>5</sup> This probably lies near (10).<sup>4</sup>

precipitin, anaphylactic antibodies, and skin hypersensitiveness to horse serum appeared as a rule toward the termination of the serum sickness. It was considered that the excessive production of antibodies followed the cellular reaction which was made evident by the serum sickness. In two of the cases that were studied, serum disease did not follow the injection of horse serum and anti-bodies did not appear in the circulation. The reason for this variation in susceptibility, a condition that has long been puzzling, has not been satisfactorily explained.

The object of the present investigation was to determine, if possible, whether the presence of antigen, namely, horse serum, bore any relation to serum disease or to the production of antibodies. It is known that by the method of specific precipitation a reaction for horse serum may be obtained in the blood, both of animals and of human beings, for many days after the injection of horse serum. Since reactions for horse serum cannot be obtained in the urine, it is improbable that horse serum is secreted as such by the kidneys.

The method employed has been to estimate by means of specific precipitin reactions the presence of horse serum at given intervals after its injection into human beings for therapeutic purposes in pneumonia and at the same time to follow the appearance and curves of precipitin for horse serum. Preliminary observations on rabbits showed that when 5 c.c. of horse serum per kilo body weight was injected intravenously, reactions for horse serum could be obtained in the blood of the rabbit over a period of from 7 days to three weeks. Precipitin for horse serum appeared in from 6 to 10 days and persisted for from 4 to 7 weeks. It is well known that animals differ widely in their ability to form antibodies, and observations on six rabbits showed considerable variation in the rate of precipitin formation, and in the duration of the reaction for horse serum in the circulation.

By this same method we have studied 14 individuals who have received from 100 to 500 c.c. of antipneumococcus horse serum intravenously. An analysis of these cases shows that they fall into two groups: In one group of eight cases the curve of precipitin formation and the persistence of horse serum was much the same as that observed in rabbits. The persistence of

the reaction for horse serum was somewhat longer, 18 to 39 days, and the appearance of the precipitins slightly slower than in rabbits, since they were not obtained in human beings until the 6th to the 15th day after injection. The injection of horse serum in all these cases was followed by severe serum disease, lasting from 11 to 28 days, and as a rule the precipitins appeared first or their concentration increased markedly towards the end of the serum disease. On the other hand, the reaction for horse serum diminished rapidly towards the termination of the serum disease and disappeared shortly thereafter. The intensity of the serum disease and the persistence of the reactions for horse serum in the circulation bore no direct relationship to the amount of serum injected.

In the second group were five cases to whom 300 to 500 c.c. of horse serum were given. In this group the results differ entirely from those obtained in rabbits and in the patients of the first group. One characteristic of these cases is the persistence in the blood of the reaction for horse serum over a very long period of time which ranged from 49 to 67 days. Practically all of these patients were lost to observation before negative reactions for horse serum were obtained. Secondly, the precipitin formation was either of extremely short duration or entirely absent. In the third place, these patients either had very mild serum disease lasting from one to five days or had none at all.

There was one other case receiving 630 c.c. of serum who showed the persistence of the reaction for horse serum in the circulation for over 75 days; he also showed precipitins and he had severe serum disease lasting 12 days. This is an exception to the cases in both groups.

An analysis of these observations shows that the reaction for horse serum disappeared fairly promptly from the blood of individuals who had severe serum disease and who formed precipitins towards horse serum, and that the disappearance of the antigens, that is the horse serum, came shortly after the subsidence of the serum disease, and when the precipitins were present in the largest concentrations. On the other hand, in the second group the reaction for horse serum persisted in the blood over long periods of time. In these patients the precipitin reaction was absent or slight and serum disease was absent or of very mild type.

It is known that individuals vary within very wide limits in their reactions to injections of foreign proteins. These observations suggest that those individuals who are relatively insusceptible have some protective mechanism either in the serum or in the cells of the body which prevents or delays the union of the antigen, in this case horse serum, with the cells of the body.

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74 (1534)

**The determination of calcium in blood and plasma.**

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The method consists in a direct precipitation of the calcium without removal of the blood proteins. The only reagents necessary are ammonium chloride (to prevent the precipitation of magnesium), and ammonium oxalate, both adjusted approximately to  $P_H$  7.4.

*Blood.*—Citratd blood (5 c.c. in a 50 c.c. centrifuge tube), is hemolyzed by the addition of four volumes (20 c.c.) of warm water and after standing 15 to 20 minutes is centrifuged to remove the stroma. An aliquot (20 c.c.) of the clear, red liquid is transferred to a 50 c.c. centrifuge tube. One c.c. of 5 per cent. ammonium chloride is added and, after mixing, 3 c.c. of 3 per cent. ammonium oxalate are added. The oxalate should be added slowly and the contents of the tube must be well mixed. After standing at least 16 hours (the much shorter time recently reported by de Waard<sup>1</sup> results in an incomplete precipitation of the calcium), the mixture is centrifuged and the supernatant liquid siphoned off. The tube is washed by the addition of 25–30 c.c. of cold water

<sup>1</sup> de Waard, D. J. *Biochem. Z.*, 1919, 97–98, p. 186.