

4685

Effect of Endothelial Blockade on the Rate of Intravenous Denaturation of Foreign Proteins.

T. H. BOONE. (Introduced by W. H. Manwaring.)

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Intravenously injected horse proteins are retained quantitatively in the normal canine circulation for at least 4 days, by which time they are so far denatured as to call forth no recognizable anaphylactic reaction on massive blood transfusion into horse-protein-hypersensitive recipients.¹

No anaphylactic denaturation is demonstrable at the end of 4 days in endothelial blockaded (India ink) dogs. Approximately half of the routine protein dose remains anaphylactically active as late as the ninth day in these animals, and about an eighth as late as the fourteenth day.

This paper summarizes the results from 20 transfusion tests. The technique has been previously described.¹

4686

A Study of the Bacterial Flora of Organs and Body-Fluids at Necropsies.

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During the past year a careful systematic bacteriological study has been made upon the organs and body-fluids that were obtained from 168 necropsies. This communication is a preliminary statement concerning the results obtained and the technique employed.

The technique for the collection of the material from the organs and body-fluids consists of carefully searing the surface of the organ or vessel and removing a portion of the tissue. The material is immediately placed in a sterile receptacle and removed to the laboratory where various kinds of media are inoculated. The media used routinely for every organ and fluid is (1) a surface infusion agar plate that contains 5% rabbit blood; (2) infusion broth; (3) Hol-

¹ Manwaring, W. H., *et al.*, *J. Immunol.*, 1927, xiii, 357, and 1928, xv, 351.

man's Meat Media and (4) Sabouraud's agar. In addition, for each fluid specimen obtained, 2 pour agar plates are prepared for the purpose of a quantitative estimation of the organisms present. Direct smears that have been stained by a special bacteriological stain have recently been added as a routine procedure for every organ or fluid cultured. This is to serve as a microscopic check for the cultured findings.

The bacteriological studies were made upon necropsies from many different pathological conditions, including deaths from acute and chronic causes and several deaths due to accidents. There was, in 75% of necropsies, an average of one to 5 hours elapsing between the time of death and the time of culturing. Very few necropsies had an interval of 24 to 48 hours before culturing. Of these, it is surprising to find that the greater portion were free of organisms.

TABLE I.
Frequency of Occurrence of Bacteria in Organs and Body-fluids in 168 Necropsies.

Name of organ or body-fluid	Total number of cultures	Per cent positive for bacteria
Bronchi	100	100
Lungs { R	122	95
{ L	94	94
Kidney	109	83
Pleural fluid	30	83
Liver	124	77
Pericardial fluid	40	73
Spleen	117	71
Heart blood	148	57
Peritoneal fluid	47	55
Spinal fluid	84	56
Urine	60	50

TABLE II.
The Frequency of Occurrence of Bacteria Isolated from 168 Necropsies.

Type of Organism	Total Number of Necropsies Positive	Per cent of Necropsies Positive
<i>Staphylococcus— aureus or albus</i>	101	60
<i>Bacillus coli</i>	91	54
<i>Streptococcus— non-hemolyticus or viridans</i>	71	42
<i>Streptococcus hemolyticus</i>	55	33
<i>Bacillus influenzae</i>	47	28
<i>Micrococcus zymogenes</i>	31	18
Pneumococcus Type IV	26	15
Diphtheroids	18	11
<i>B. lacti-aërogenes</i>	17	10
<i>Bacillus proteus</i>	10	6
Pneumococcus Type III	9	5
Pneumococcus Type I	7	4
Pneumococcus Type II	6	4
<i>Bacillus Friedländer</i>	5	3

TABLE III.
Frequency of Occurrence of Bacteria Isolated from Each Organ and Body-fluid.

	Name and Total Number of Organs and Body-fluids Cultured.												
	Heart blood	Peri-cardial fluid	Lungs		Bronchi	Pleural fluid	Liver	Bile	Spleen	Kidney	Urine	Peri-toneal fluid	Spinal fluid
			Right	Left									
	148	40	122	94	100	30	124	75	117	109	60	47	84
	%	%	%	%	%	%	%	%	%	%	%	%	%
<i>Staphylococcus aureus</i> or <i>albus</i>	11	23	40	47	52	33	14	11	15	21	15	21	19
<i>Bacillus coli</i>	14	20	36	26	25	0	27	16	19	38	23	21	8
<i>Streptococcus non-hemolyticus</i> or <i>viridans</i>	6	10	27	21	32	17	10	3	9	13	3	11	4
<i>Streptococcus hemolyticus</i>	9	15	21	24	26	10	10	0	13	9	2	6	6
<i>B. influenzae</i>	1	3	22	30	32	7	2	0	1	0	0	2	2
<i>Pneumococcus</i>	3	5	5	6	3	7	2	0	1	0	0	2	1
Type I	3	0	4	5	4	7	2	0	2	3	0	0	0
Type II	3	0	4	5	6	3	1	0	0	0	0	0	5
Type III	4	3	17	14	17	10	2	0	3	2	0	0	4
Type IV	3	0	6	4	5	3	3	5	5	6	5	2	1
<i>B. lacti-aërogenes</i>	3	0	2	3	3	0	1	1	1	0	2	0	0
<i>B. Friedländer</i>	1	0	2	2	3	3	5	1	1	4	2	0	1
<i>B. proteus</i>	1	1	3	2	2	0	3	0	4	5	0	0	0
Diphtheroid	1	1	3	2	2	0	3	0	4	5	0	0	0
<i>M. zymogens</i>	5	3	2	2	1	0	7	8	6	3	5	0	4

However, it was found that if there were 7 to 9 hours elapsing between time of death and time of culturing there would be an increase in the frequency of the occurrence of anaerobic organisms within the tissues. The organism that predominated was a large gram positive spore-forming rod which resembles *Bacillus sporogenes* in many of its cultural characteristics. Yeasts and molds were absent from the cultures, except from lungs and bronchi. From this source, the green mold was commonly present.

No attempt will be made at this time to offer an interpretation of the results presented above. The work is being continued and it is our hope that after a larger number of necropsies have been investigated, we may be able to draw some significant conclusions.

4687

The Effect of Roughage Upon Growth.

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The diets shown in Chart 1 were designed for the purpose of determining the influence of large quantities of "roughage" upon the rate of growth. Ration 1 contains 10% cellulose while numbers 2 and 3 contain 20%. Diet 4 contains 20% of agar-agar. Nos. 1 and 2 contain moderate amounts of fat while numbers 3 and 4 have 45% of their total weight in the form of lard and cod liver oil. All rations were designed to contain adequate amounts of protein, fats, carbohydrates, mineral matter and vitamin supplements. The levels for protein and mineral matter were increased for rations 3 and 4 in accordance with the procedure of Smith and Carey.¹ The salt mixture employed was that of Osborne and Mendel. Cellophane was selected as a source of cellulose since it represents a very pure form. In addition to the mixed diet all animals were fed separately a daily allowance of 200 mg. of vitavose and 3 drops of cod liver oil.

Ten male rats were reared from the time of weaning upon rations 1 and 2. These showed a marked decreased rate of growth when compared with a control group whose growth rate is shown by curve C while representative rates for the other groups are shown by typical curves properly numbered. The control ration was iden-

¹ Smith, A. H., and Carey, E., *J. Biol. Chem.*, 1923, lviii, 425.