were added to 5 gm. quantities of the basal diet, and the food was placed in a vial with 20 two-hour-old larvae. The vials were kept in a temperature cabinet at 32°C. and 70% humidity and the average time of pupation recorded. A vitamin B_1 concentrate prepared from rice polishings was used. The average time required for pupation with the various supplements to the basal diet were: vitamin B_1 concentrate equivalent to 50% rice polish no pupation, 5% autoclaved yeast no pupation, 1% autoclaved yeast plus vitamin B_1 concentrate equivalent to 2% rice polish 19.2 days. This indicates that Tribolium requires a heat stable factor in addition to vitamin B_1. It was found that this heat stable factor was destroyed by autoclaving at pH 13 for 4 hours.

With 1% autoclaved yeast to supply the heat stable factor the larvae responded in a quantitative manner to the addition of the vitamin B_1 contained in small amounts of rice polish as follows: with 0.1% rice polish average time of pupation was 20.3 days; 0.05% rice polish, 23.8 days; 0.025% rice polish, 40.2 days. With 1% rice polish as the sole addition to the basal diet the average time of pupation was 98 days.

8152 C

Positive Flocculation Tests in Rabbits Inoculated with Flocculate from Human Syphilitic Serum.

F. RYTZ. (Introduced by W. P. Larson.)

From the Clinical Laboratories of the Minneapolis General Hospital, Minneapolis, Minnesota.

In an effort to determine the nature of flocculate obtained from human syphilitic serum, by means of flocculation tests for syphilis, an attempt was first made to produce precipitins in rabbits by intravenous inoculations with such flocculate. Attempts in dissolving the flocculate were at the time unsuccessful. A rabbit was therefore inoculated with flocculate suspended in sterile saline. After 5 intravenous injections the rabbit serum was tested for precipitin formation and found negative in various dilutions. On the other hand, the serum from that rabbit, when added to 2 plus positive syphilitic serum, changed the positiveness to a strong 4 plus reaction.

An attempt was then made to produce in rabbits, by inoculation

of flocculate from human syphilitic serum, the antibodies causing flocculation of antigen in syphilitic serum.

In this experiment 16 albino rabbits were used, 10 males and 6 females. The flocculation test employed was that originated in the laboratories of the Minneapolis General Hospital.[1] This was supplemented by the Wassermann reaction as an extra check and as a special precaution. The latter test was performed by a technician who had no knowledge of the source of the blood samples involved. After the rabbits proved to have negative flocculation tests, they were inoculated intravenously with from 16 to 60 units of flocculate from the M.G.H. test or from the Kahn test. The flocculate was obtained from human syphilitic serum which proved to be 4 plus positive by M.G.H., Kahn and Wassermann methods. The flocculate from one 4 plus reaction was called 4 units. Before injection, the flocculate was centrifuged and washed 3 times in sterile saline. The dose was finally suspended and shaken in 5 cc. of sterile saline. At intervals of 5 days the animals were thus injected intravenously, with increasing doses, from 2 to 5 times. The last 2 injections consisted, except in the case of 3 of the animals, of 120 units of flocculate each.

After 2 to 3 inoculations all of the animals showed positive flocculation tests, varying in positiveness from 2 to 4 plus. In most of the animals 4 inoculations resulted in strong 4 plus reactions, and 4 animals gave reactions from 9 plus to 20 plus by quantitative method. In most of the cases the final test was performed 8 days after the last injection.

Two control animals were injected, in the same manner and in the same proportion as the test animals, with antigen emulsion alone. The serum from these control animals remained negative throughout the experiment.

Of the 14 rabbits inoculated with flocculate, 11 were given flocculate from our own test, and 2 received flocculate from the Kahn test. No difference was noted in potency of the flocculate from the 2 tests.

One animal, rabbit No. 4, was injected with flocculate from the serum of rabbits No. 1 and No. 3. After 3 intravenous injections its serum was 3 plus positive. The fourth injection, 100 units of flocculate, was given under ether anesthesia. A week later the positiveness of that rabbit had dropped from 3 plus to 1 plus. It was then given another injection of 100 units of flocculate from the serum of rabbit No. 3. Eight days later the positiveness had risen

[1] Rytz, F., *Am. J. Clin. Path.*, 1935, **5**, 159.

TABLE I.

No. of flocculate units injected and resulting positiveness of each rabbit. The serum unit for the flocculation test is 0.15 cc. The quantitative unit is the amount necessary for a 4 plus reaction.

Rabbit No.	No. of injections	Total No. of floc. units	Flocculat. reaction	Wassermann reaction	Remarks
1	5	408	20+	4+	One-fifth unit of serum gave a 4-plus reaction. Showed strongly 4-plus flocc. reaction 2 months after the last injection.
2	2	48	2+	neg.	Developed snuffles. Died.
3	3	136	4+	4+	" " "
4	5	344	3+	neg.	Injected with flocculate from rabbits 1 and 3. Ether anesthesia reduced the positiveness to 1-plus. Positiveness returned later to 2-plus.
5	4	360	4+	4+	Positiveness 2-plus 3 days after ether anesthesia.
6	4	360	4+	4+	
7	4	360	3+	2+	
8	4	360	3+	4+	
9	4	360	16+	4+	Injected with flocculate from the Kahn test. One-fourth unit of serum gave a 4-plus flocc. reaction.
10	4	360	4+	4+	Injected with flocculate from the Kahn test.
11	4	360	9+	4+	One-third unit of serum gave 3-plus flocc. reaction.
12	4	360	4+	4+	
13	4	360	12+	4+	One-fourth unit of serum gave 3-plus flocc. reaction.
14	4	360	3+	1+	
15	4 antig. emuls.		neg.	neg.	Injected with 4.5 cc. of antigen emulsion only.
16	4 " "		"	"	" " " " " " " " " "

from 1 plus to 2 plus. Two months later the serum of this rabbit was still 2 plus positive.

Rabbit No. 5, after showing a 4 plus reaction, was also anesthetized and kept under ether anesthesia for 5 minutes. Three days later the positiveness of its serum had dropped from 4 plus to 2 plus. No further injections were given to that rabbit.

After rabbit No. 9 had given a final reaction of 16 plus, it was killed by cutting the jugular vein. Fifteen cc. of clear serum was thus obtained which yielded 1,620 units of flocculate. The serum, therefore, contained 1,260 units of flocculate above the original 360 units injected.

Conclusions. (1) For lack of a better name, the antibodies causing flocculation of antigen in human syphilitic serum might be termed "flocculins". (2) It has been shown that flocculate obtained from human syphilitic serum, by means of flocculation tests for syphilis, will cause positive flocculation tests in rabbits when inoculated intravenously. Such reactions will remain positive for at least 2 months after the last injection. Rabbits inoculated with antigen emulsion alone do not develop positive flocculation tests. (3) Apparently, the serological factors involved in the formation of "flocculins" in the animal body are influenced by ether anesthesia. (4) Flocculate obtained from the serum of a rabbit injected with flocculate from human syphilitic serum, will cause the formation of "flocculins" in another rabbit when injected intravenously. (5) The serum of a rabbit inoculated with a definite number of flocculate units, may develop many more units than were thus injected.

8153 C

Intra-Enteric Pressure in Experimental and Clinical Intestinal Obstruction.*

LOUIS SPERLING, JOHN R. PAINE, AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

As a preliminary to a series of experiments on the effect of increased intra-enteric pressure upon the physiology and structure of the bowel wall, occasion was taken to measure the levels of sustained pressure which obtained in the intestine of dogs with low ileal obstruction.

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