THE QUESTION OF SENSITIZATION OF JOINTS WITH NON-HEMOLYTIC STREPTOCOCCI.

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These experiments were planned in an attempt to substantiate the hypothesis that the arthritis of acute rheumatic fever is the result of a preliminary specific sensitization of the joints by non-hemolytic streptococci. This hypothesis has been advanced by both Herry (1) and Faber (2), and accepted by certain authors as the most probable explanation of the peculiar type of inflammation found in the joints of patients with this disease.

Herry claims that the peculiarity of the rheumatic lesions is due to an "*endotoxine rhumatismale*" which, he states, he was able to prepare from streptococci grown on solid media by a process of drying, grinding, and extracting with normal saline, and finally removing the bacterial bodies by centrifugalization. He states that the extract from 2 mg. of streptococci was fatally toxic for rabbits, and that the introduction of a few drops of clear extract into the joints of rabbits sensitized these structures so that intravenous inoculation of the animals with homologous streptococci after 8 to 15 days was constantly followed by arthritis in the sensitized joints.

In view of the difficulty experienced by most workers in obtaining endotoxins from any type of streptococci, the claim of Herry that he could produce a powerful toxic substance from a small amount of non-hemolytic streptococcus culture is most surprising. Faber, indeed, was unable to repeat successfully his experiments; and we have likewise failed to obtain an endotoxin from these cocci. Faber, on the other hand, reported that by injecting rabbits' knees with killed cultures of non-hemolytic streptococci these joints were rendered more liable to involvement following subsequent intravenous inoculation with homologous living microorganisms. He, unfortunately, does not state whether the "sensitized joints" were the only joints involved. Because preliminary injection of rabbits' knees with killed pneumococci or typhoid bacilli did not render these structures sensitive to a subsequent intravenous inoculation of the animals with non-hemolytic streptococci, he claimed that "this preparatory or sensitizing process is, within narrow limits, a strictly specific one." It should be pointed out that in his experiments arthritis did not occur in animals first injected

into the joints with killed pneumococci or typhoid bacilli and subsequently inoculated intravenously with homologous living bacteria. If the pneumococcus-treated animals are eliminated from his protocols, cross-sensitization was obtained in only two out of four rabbits instead of two out of ten.[1] This makes it appear that the so called *sensitization* in his experiments was the production of a local lesion which upon healing left a *locus minoris resistentiæ*, a place favorable for the localization of bacteria having a tendency to produce joint lesions. When pneumococci or typhoid bacilli, having little tendency to produce arthritis, were inoculated intravenously into rabbits with joints previously injected this *locus minoris resistentiæ* was not made evident. This seems to us the most probable explanation of Faber's experiments. It is not sufficient, however, to explain the fact that his rabbits inoculated intravenously three times with "Streptococcus 7" had arthritis more frequently than those inoculated only once or twice. We think it most probable that small areas of inflammation were set up in the joints of rabbits following the first or second inoculation, and that these small lesions produced points of lessened resistance. Then, if another strain of streptococci had been used for the third inoculation, joint lesions would have been expected with the same frequency as if the homologous strain had been introduced.

Another way to determine this point would be to inject the right knees of several rabbits with one strain of streptococci and the left with another, and subsequently to inoculate half of the animals with one of the strains and the remainder with the other. If arthritis occurred only in the knees of animals in which the same strain was used for both intraarticular injection and intravenous inoculation one might conclude that specific joint sensitization had been induced. If, on the other hand, arthritis occurred in both knees or in other joints, the probability that specific sensitization had taken place would be seriously doubted. In this manner we attempted to elucidate the question.

EXPERIMENTAL.

Method.

In all instances the experimental animals were brown rabbits weighing 1,200 to 2,000 gm. The hair over the knees was clipped closely so that the bony prominences could be easily recognized and the joints minutely inspected. In order to have a more accurate record of the degree of swelling of the knees these joints were measured daily as follows: the widest distance between the internal and external condyles of the femora, and the broadest portion of the upper end of the

[1] Faber (2), Table VI.

tibiæ. As these measurements were over bony prominences it was fairly easy to have them comparable from day to day. They were made with special calipers having a vernier scale reading to 0.1 mm. It may be thought that such measurements are too fine for practical purposes and that conclusions based on them are liable to be faulty, but considerable preliminary work satisfied us that repeated measurements on normal animals are constant to within 0.2 to 0.5 mm., and frequently there is practically no variation; hence it seemed justifiable to record the degree of joint injury in this manner. Notes were also made of redness, swelling, heat, and stiffness. The last symptom, when occurring early, was regarded as a measure of pain; when occurring later, as an indication of the amount of permanent injury. For the first 5 or 6 days following the intraarticular injections the animals were inspected daily, then every 2nd day for about 10 days, and subsequently twice a week until they were inoculated intravenously; then daily inspections were resumed.

In the first three experiments, after preliminary measurements of the knees, these joints were injected with killed streptococci in the amounts indicated in the protocols; the suspensions were prepared as follows: The centrifugates of 18 to 24 hour broth cultures of the cocci were suspended in normal saline and heated ½ hour at 56°C., then tested by plating to insure sterility. The suspensions were so diluted that 0.5 cc. contained the desired amount of streptococci. Most of the joints were injected with an amount corresponding to 0.5 cc. of the original broth culture, as it had been determined in previous experiments that this amount of heat-killed, non-hemolytic streptococci could be injected into the knee joints of rabbits without demonstrable permanent damage. The injections were made according to the method described by Faber; the needle was passed in a proximal direction through the middle of the patellar tendon until it was felt to slip into the bursa under the patella; the suspension was then slowly introduced, a small amount withdrawn into the syringe by suction and reinjected (piston test) in order to be sure that the needle was properly placed; the needle was then quickly withdrawn to prevent leakage into the periarticular tissue. When it was certain that the bacteria had been injected into this tissue the animals were discarded. The bacteria for intravenous inoculation were usually grown for 18 hours in 10 per cent rabbit serum broth, centrifugalized, and resuspended in sterile Ringer's solution. Except with the first culture of Strain Z75, the streptococci had been recently passed several times through rabbits' knees in an attempt to increase their virulence.

The streptococci were all isolated from blood cultures of patients with rheumatic fever, were typical *viridans* strains possessing different biochemical and immunological characteristics, and had decreasing degrees of virulence as follows: A49, A135, 38D, and Z75.

At the postmortem examination the degree of joint involvement was noted, and films from all the joints were prepared and examined microscopically. In some instances there seemed to be an increased amount of clear fluid, but films revealed no exudation of cells; hence these joints were considered normal. Four degrees of inflammation were distinguishable.

Degree.	Gross appearance.	Microscopic appearance.
+++	Yellow, turbid, purulent fluid.	Marked increase of exudative cells.
++	Gray, cloudy or hazy fluid.	Distinct to marked increase of exudative cells.
+	Increased clear fluid.	Distinct increase of exudative cells.
±	Normal amount, clear fluid.	" " " " "

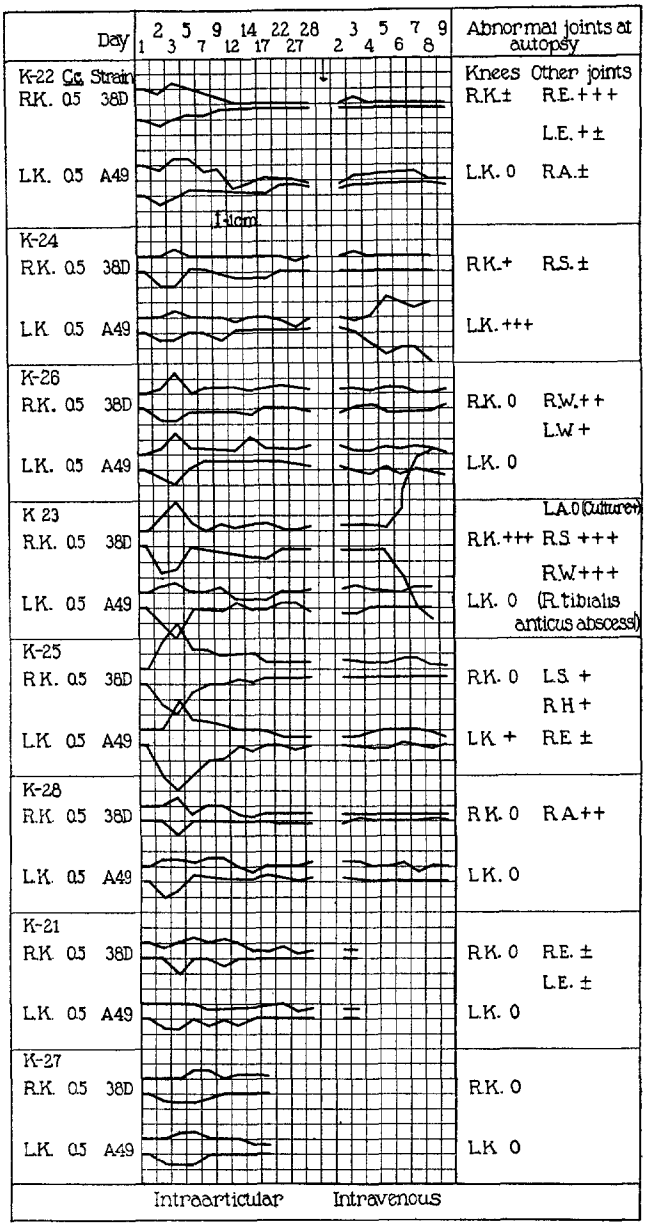
Except in Experiment 4, half of the rabbits received sodium salicylate by stomach tube from the day previous to intravenous inoculation until the time of death. This was done to study the effect of salicylates upon possible joint sensitization, for if bacterial sensitization is the important factor in determining the localization of rheumatic arthritis it would seem probable that salicylates would exert a profound antiarthritic influence. In another communication (3) we have shown that sodium salicylate apparently decreases the intensity of the arthritis in some rabbits inoculated intravenously with non-hemolytic streptococci.

If one considers only the knee involvement among the animals in Experiments 1 and 2, here reported, it would seem as though the salicylates might have exerted an inhibitory influence upon joint sensitization; but consideration of all the inflamed joints makes this less probable.

Explanation of Text-Figures.—The upper line for each knee shows the change in the measurement of that joint through the condyles of the femur; an upward direction of the line indicates increase, a downward direction, decrease. The lower line for each knee shows a change in measurement through the broadest portion of the upper end of the tibia; but here a downward direction of the line indicates an increase, and an upward direction, decrease. The area between these two lines indicates, therefore, the variation in the size of the knees. Broken lines indicate that no measurements were made on that day.

In each chart the blank space between the two periods of observation indicates that this was the last day of the "intraarticular period" and the first of the "intravenous."

R. K. indicates right knee; L. K., left knee; other joints are designated in a similar manner by the initial of the side and joint.



TEXT-FIG. 1. Measurements of knees following intraarticular injection and intravenous inoculation. The arrow indicates the day on which the animals received an intravenous inoculation of the sediment of 9.3 cc. of Streptococcus 38D/4/2.

Experiment 1.—The knee joints of eight rabbits were injected with the sediment of 0.5 cc. of broth culture of killed streptococci as follows: right, with Strain 38D/3/4;[2] left with Strain A49/8/4. The animals were observed for 4 weeks, when it was evident that the inflammation resulting from the intraarticular injection had entirely subsided. 17 days after this first injection, Rabbit K-27 died, evidently from an intestinal infection; both knees appeared normal, and the synovial fluids were free from cellular exudate. This confirms the conclusion derived from clinical observation of the knees, that the injury resulting from the introduction of killed streptococci into them did not persist more than 3 weeks. Salicylate treatment was started with Rabbits K-21, K-23, K-25, and K-28 on the 27th day, and on the next day each of the seven surviving animals was inoculated intravenously with the sediment of 9.3 cc. of serum broth Culture 38D/4/2. The animals were then inspected daily until the 7th, 8th, or 9th day when they were sacrificed and autopsied. Films and cultures were made of all the joint fluids (Text-fig. 1).

Text-fig. 1 shows graphically the reaction of the knees following both the intraarticular injections and the intravenous inoculations and also the degree of inflammation in all abnormal joints at the time of autopsy. Although the animals responded differently to the intraarticular injection, each animal showed a similar amount of swelling in the two knees. It seems evident, therefore, that the two strains of streptococci, when killed, were equally irritating. This is noteworthy when compared with the results obtained from inoculating the two strains intravenously; Strain A49 is much more virulent than Strain 38D. They have different fermentation reactions and are distinct immunologically. One would think, therefore, that if joints were specifically sensitized by having previously reacted to one strain, the intravenous inoculation with that strain would always result in a recurrence of arthritis in that joint, and the opposite knee having previously reacted to the other strain would remain free from a second reaction. This expected result, however, was not obtained in this experiment. Rabbit K-21, dying 2 days after the intravenous inoculation from a Gram-negative bacillus infection, showed no evidence of involvement of either knee, but had slight arthritis in both elbows. Among the six animals surviving the full period of the experiment there were three inflamed

[2] The first figure indicates the strain of streptococcus; the second, the number of passages through rabbits' joints; and the third, the number of subcultures since the last animal passage.

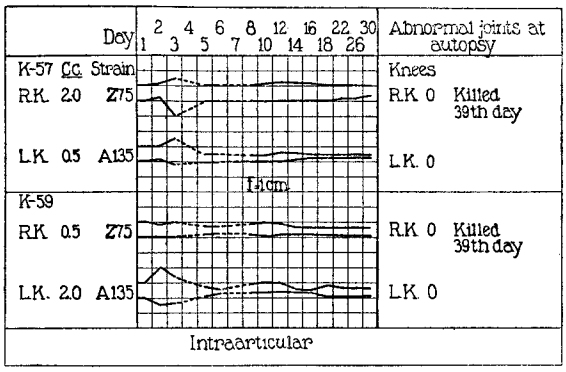
right knees,—the joints injected with the same type of bacteria subsequently used for intravenous inoculation; there were also two inflamed left knees,—the ones previously injected with the other strain; and, in addition, there were twelve other inflamed joints among these animals; also one joint, showing no cellular reaction, from which a pure culture of Streptococcus 38D was grown; this must be considered evidence of bacterial invasion of the ankle in this rabbit (No. K-23). In the seven animals, twenty joints were, therefore, involved, but only three of these were previously injured with the same type of streptococcus that was used for intravenous inoculation. Four knees so injured failed to show any abnormalities post mortem. The knees of one animal (No. K-25) appeared slightly stiff for 3 days after the intravenous injection; this stiffness was thought to be a residuum from the inflammation following the initial joint injury. Both knees of this rabbit showed a marked reaction to the intraarticular injection, but the left, first injured by Streptococcus A49, was the one invaded following the intravenous inoculation with Streptococcus 38D, and three other joints of this animal were also acutely inflamed. Rabbits K-22 and K-23 might be cited as examples of specific joint sensitization if only the knees were considered, but the involvement of three other joints in each animal renders this position untenable.

There seemed to be slight, if any, relationship between the amount of gross reaction following the initial joint injury and the subsequent localization of the living bacteria. By this we do not mean to imply that severe injury to a joint would not be a factor in determining localization of microorganisms in that joint, but rather that the amount of local injury resulting from the intraarticular injection in the rabbits was too slight to have unfavorably influenced the reacting power of the knees. It is probable that only by producing such slight injuries is it possible to study the question of bacterial joint sensitization, for if more severe trauma were inflicted a *locus minoris resistentiæ* would result and doubtless favor the localization of many types of microorganisms circulating in the blood stream.

The cultures from the synovial fluids of all the joints were sterile, with the exception of those from the right knee and the left ankle of Rabbit K-23. The upper half of the right tibialis anticus, and extensor longus digitorum muscles of this animal were markedly inflamed and

yielded a small amount of purulent material on incision. These findings indicate that the animal had comparatively little natural resistance to the infection.

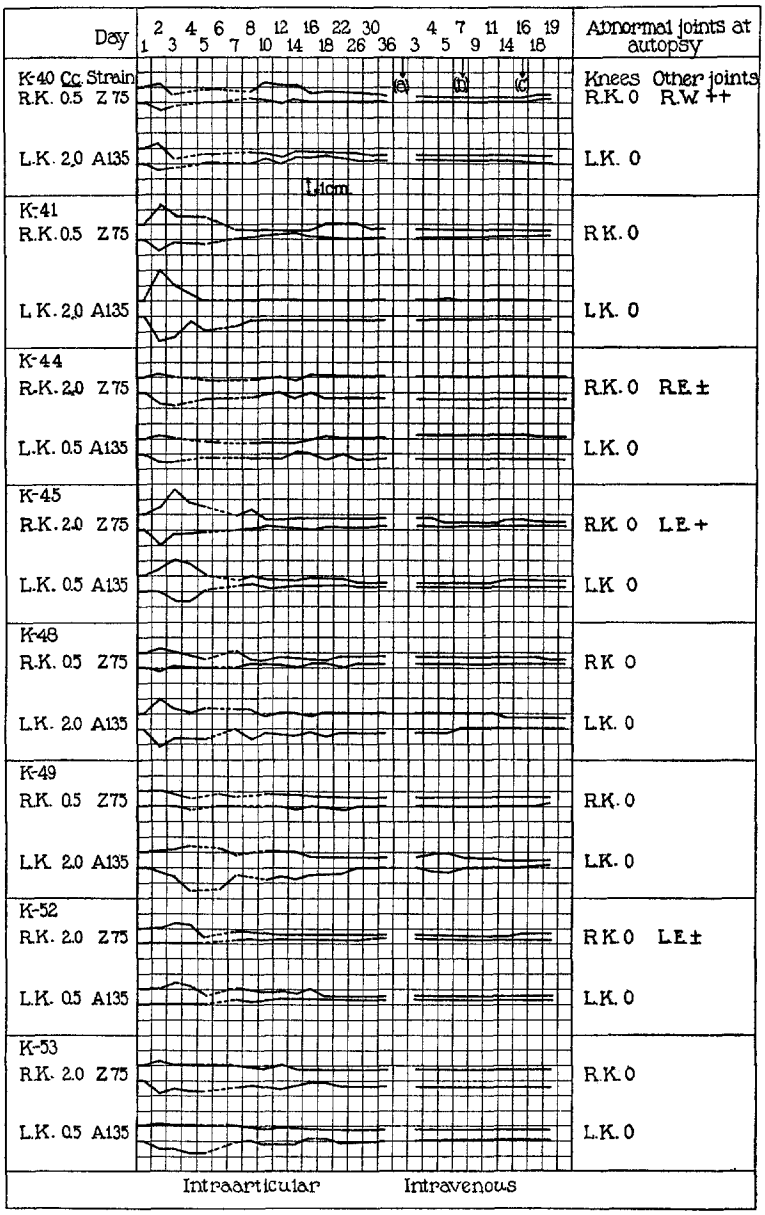
Experiments 2 and 3 serve as controls for one another in that the animals received intraarticular injections of the same streptococci: Strain Z75 in the right knees, and Strain A135 in the left; in half the animals the sediment of 0.5 cc. of broth culture of Streptococcus Z75 and 2 cc. of Streptococcus A135 were used; in the other half the amounts of these two streptococci were reversed. Two animals (Nos. K-57 and K-59) were controls in both experiments; their knee



TEXT-FIG. 2. Measurements of knees of control animals following intraarticular injection with Streptococcus Z75 and Streptococcus A135.

joints were injected in the same manner as in the others and observed for 39 days; then the rabbits were sacrificed and the joints found to be normal (Text-fig. 2). In these two rabbits the knee receiving the larger amount of culture showed the greater reaction, which would be expected; but both knees of many of the animals in the two experiments showed reactions of similar intensity.

Experiment 2.—The knees of eight rabbits were injected, and observed for 36 days, when it was certain that they had recovered from the local injury. On the 35th day half the animals (Nos. K-48, K-49, K-52, and K-53) were given 0.2 gm. of sodium salicylate per kilo of body weight; this treatment was continued until the termination of the experiment. On the 36th day all the rabbits were inoculated intravenously with the sediment of 50 cc. of broth Culture Z75/0/11; 7 days later none showed any evidence of joint involvement, so they were reinoculated with the same amount of Culture Z75/1/2; 8 days later they still failed



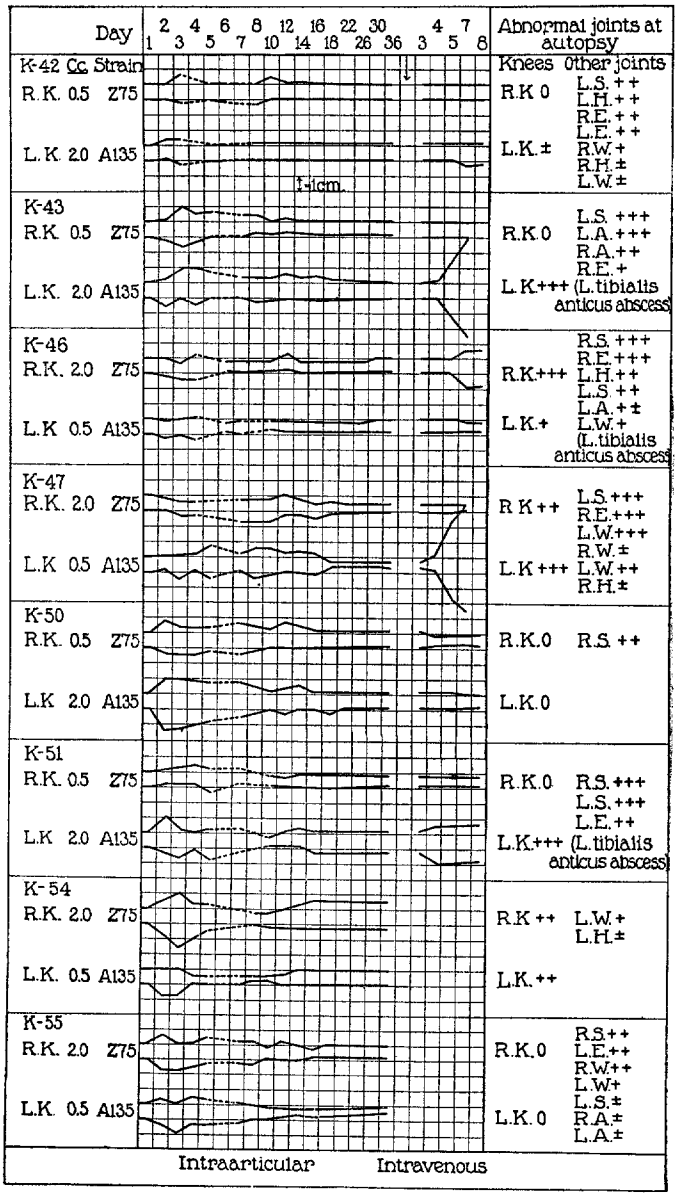
TEXT-FIG. 3. Measurements of knees following intraarticular injection and intravenous inoculation. The arrows indicate the days on which the animals received intravenous inoculations of (*a*) the sediment of 50 cc. of Streptococcus Z75/0/11, (*b*) the sediment of 50 cc. of Streptococcus Z75/1/2, and (*c*) the sediment of 50 cc. of Streptococcus Z75/3/2.

to show any effect from the two inoculations, so were similarly inoculated with 50 cc. of Culture Z75/3/2. 3 days later all appeared well and clinically free from joint lesions; they were sacrificed and examined in the usual manner. The results are shown in Text-fig. 3.

Although most of the injected knees showed some degree of reaction following local injury, none gave any evidence of arthritis following three intravenous inoculations of the animals with large amounts of Streptococcus Z75. Among the eight rabbits, only four joints were found to be definitely diseased. This proved, however, that the inoculum, in the large amounts employed, was sufficient to incite arthritis. The primary injury produced in the right knees of all the animals by injection of this coccus would seem to furnish perfect conditions for the demonstration of specific joint sensitization. The observation that rabbits have more arthritis following repeated intravenous inoculations with non-hemolytic streptococci of low virulence is claimed by Faber to furnish further proof of joint sensitization. Both of the above mentioned conditions, *viz.* intraarticular injection of cocci and repeated intravenous inoculation, were fulfilled in this experiment without any evidence of such sensitization. It will be noted that the salicylated animals had fewer abnormal joints than the non-salicylated; this may be advanced as an argument in favor of eliminating these four animals from consideration, but lack of inflammation in any of the knees of the other four animals, and the results with salicylated animals in other experiments seem of sufficient weight to warrant the inclusion of all in this report.

Experiment 3.—Eight animals were used in this experiment; the knees were injected in the same manner and with the same cultures as those of Experiment 2. On the 35th day half of them (Nos. K-50, K-51, K-54, and K-55) were started on sodium salicylate. On the 36th day all were inoculated intravenously with the sediment of 30 cc. of serum broth Culture A135/2/2. The following day two of the salicylated rabbits (Nos. K-54 and K-55) died; the rest survived for 7 or 8 days, when they were sacrificed and examined as usual. The results are shown in Text-fig. 4.

Streptococcus A135 was much more virulent than Streptococcus Z75 and, therefore, furnished another type of experimental condition. In the entire group there were forty-five abnormal joints, an average of 5.6. The two rabbits (Nos. K-54 and K-55) dying the day after



TEXT-FIG. 4. Measurements of knees following intraarticular injection and intravenous inoculation. The arrow indicates the day on which the animals received an intravenous inoculation of the sediment of 30 cc. of Streptococcus A135/2/2. Rabbits K-54 and K-55 died the following day.

the intravenous inoculation had eleven inflamed joints, an average practically the same as that which prevailed in the surviving group, so it seems justifiable to include them in the general discussion. All the knees injured by intraarticular injection seemed to have recovered completely by the end of the 3rd week; at least, there was no appreciable change in size after that time. There was no constant relation between the reaction of the knees to intraarticular injection and the liability for any given joint to be involved after the intravenous inoculation; for example, both knees of Rabbit K-50 showed the most severe

TABLE I.

Summary of Joints Involved in Experiment 3.

Joint.	Rabbit No.								Total involved.	Per cent involved.
	K-42	K-43	K-46	K-47	K-50	K-51	K-54	K-55		
R. S.	0	0	+	0	+	+	0	+	4	50
L. S.	+	+	+	+	0	+	0	+	6	75
R. E.	+	+	+	+	0	0	0	0	4	50
L. E.	+	0	0	+	0	+	0	+	4	50
R. W.	+	0	0	+	0	0	0	+	3	37
L. W.	+	0	+	+	0	0	+	+	5	62
R. H.	+	0	0	+	0	0	0	0	2	25
L. H.	+	0	+	0	0	0	+	0	3	37
R. A.	0	+	0	0	0	0	0	+	2	25
L. A.	0	+	+	0	0	0	0	+	3	37
R. K.	0	0	+	+	0	0	+	0	3	37
L. K.	+	+	+	+	0	+	+	0	6	75

and persistent reaction, and neither of them was diseased at the time of death. From this animal alone one might conclude that the preliminary articular injury had led to a condition of immunity, but the results in Rabbit K-43 point in the other direction. Both knees of this animal showed intense and fairly persistent swelling following the initial injury, but only the left was involved following the intravenous inoculation; four other joints of this animal were also inflamed, and there was an abscess in the left tibialis anticus muscle. So many foci

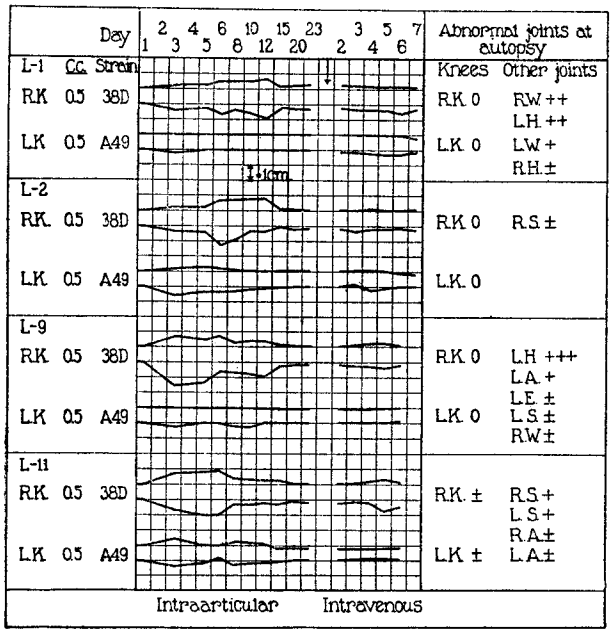
of infection indicate that the animal had much less resistance than did No. K-50.

A summary of the distribution of arthritis among the joints of the different rabbits (Table I) shows that the left knee—the one previously injected with Strain A135—was involved in six animals; the right—previously injected with Strain Z75—was invaded half as frequently. If we consider the percentage of arthritis in the various sets of joints, *i.e.* right shoulder, left shoulder, etc., it is evident that the left shoulder was involved as frequently as the left *sensitized* (?) knee, and that in most instances more joints were affected on one side of the body than on the other. Two of the animals failed, in addition, to show arthritis in either of the previously injected joints; it would, therefore, be illogical to conclude that the localization of inflammation in the left knees of the other six was due to a specific streptococcus joint sensitization. We are inclined to regard the localization in the knees as due to the same factors that determined the localization in other joints—virulence and invasive properties of the streptococci.

Because of the apparent failure to induce joint sensitization with heat-killed streptococci an attempt was made in one experiment to sensitize joints with an extract of streptococci prepared by the method used by Herry. This was considered advisable because of the theory of Zinsser (4, 5) that substances of much smaller molecular size than bacteria may penetrate cells and lead to an allergic state of these cells. It is evident that the experiment as planned by us is not of the same nature as those usually performed to demonstrate sensitization; in the latter the animal is sensitized by introduction of the antigen intravenously or intraperitoneally, possibly subcutaneously, and the local allergic state demonstrated subsequently by introducing the antigen into some particular tissue, as the skin or mucous membrane. This local hypersensitiveness is only a part of a general allergy and cannot be considered the same as the "joint sensitization" described by Herry and Faber.

Experiment 4.—An extract of Streptococcus 38D and one of Streptococcus A49 were prepared as follows: Cultures were seeded on glycerol potato agar to which rabbit blood had been added. After 18 hours the growth was carefully scraped off, taken up in a small amount of saline, and desiccated *in vacuo* over P_2O_5 or KOH. Each extract consisted of two lots; one required 48 hours for

desiccation and the other only 2 hours. The dried bacteria were then pulverized by grinding in an agate mill for 24 hours. The powder was weighed and mixed with sodium chloride solution and water in such proportions that each cubic centimeter contained 4 mg. of dried bacteria and 8 mg. of NaCl. The bacterial suspension was allowed to stand at room temperature with frequent shaking of the container for another 24 hours, when it was centrifugalized at high speed until water-clear. The supernatant solution was pipetted off; 0.5 cc. of Strain 38D was injected into the right knee of each of four rabbits, and 0.5 cc. of Strain A49 into the left. Cultures of the extracts showed that Strain 38D



TEXT-FIG. 5. Measurements of knees following intraarticular injection and intravenous inoculation. The arrow indicates the day on which the animals received an intravenous inoculation of the sediment of 5 cc. of Streptococcus A49/0/10.

was quite heavily contaminated with white non-hemolytic staphylococci, and Strain A49 contained a very few of the same contaminants. No streptococci were recovered from either solution. The local reaction induced by the intraarticular injection might, therefore, have been due to the presence of contaminating staphylococci, as well as to the streptococcus extract. Indeed, the more marked swelling of the right knees when compared with the left suggests that the heavier contamination of Solution 38D was responsible for the severe reaction. It was shown, however, by marked turbidity of the solutions on boiling that they contained a large amount of bacterial protein.

Because of the contamination with staphylococci the solutions were filtered through a Berkefeld filter, and subsequently shown to be sterile. Their toxicity was tested as follows: one rabbit was injected intravenously with 3 cc. of Strain 38D, another with 3 cc. of Strain A49, and a third with 2 cc. of each. None of these animals showed any toxic symptoms. This result would be expected from our general knowledge of the lack of endotoxin in members of the coccus group of bacteria.

23 days after the four rabbits had received intraarticular injections of the two bacterial extracts, each was inoculated intravenously with the sediment of 5 cc. of culture of Streptococcus A49/0/10. They were observed for a week, then sacrificed and examined in the usual way. The results of this experiment are shown in Text-fig. 5.

As already noted, the right knees of all the rabbits reacted quite violently to the intraarticular inoculation. This was probably due to the contamination of the extract. The left knees injected with Streptococcus A49 extract, on the other hand, reacted only mildly. Therefore, at the time of intravenous inoculation the right knee of each animal had been intensely inflamed and possibly sensitized to Streptococcus 38D, and the left knee possibly sensitized to Streptococcus A49. The intravenous inoculation with Streptococcus A49 would be expected to bring out evidence of this sensitization if it existed. The dose was fortunately proper to induce arthritis in all the animals, but only one of them (No. L-11) had inflammation of the knees. Among the four animals there were sixteen inflamed joints, and among these the wrists shoulders, hips, and ankles were affected more frequently than the knees. It is evident, therefore, that the preliminary irritation of the knees of these rabbits did not render them especially liable to localization of the streptococci. The results in general agreed with those of the previous experiments.

DISCUSSION.

A summary of all the results is given in Table II, in which the order of the experiments is arranged according to the percentage of total joints involved in each group. This shows that there was provided a fairly wide range of conditions in respect to virulence and power of the various streptococci to invade joints. In two experiments (Nos. 2 and 4) the injected knees showed less tendency to be involved than did other joints, and the knee previously injected with

killed streptococci or bacterial extract of the same strain that was inoculated intravenously reacted in exactly the same manner as the opposite knee that had been previously injected with another strain of streptococci. In another experiment (No. 1) the knees injected with homologous cocci were involved slightly more often than those injected with heterologous cocci, but the fact that the larger proportion of injected knees failed to be involved following intravenous inoculation of the animals shows that inflammation of some of the knees cannot be attributed to sensitization. In the remaining experiment (No. 3) twice as many knees injected with the homologous strain were involved

TABLE II.

Summary of the Results of All Experiments.

Experiment No.	2	1	4	3	Total.
No. of animals	8	7	4	8	27
Total No. of joints (12 per animal)	96	84	48	96	324
Total No. of joints involved	4 = 4%	20 = 24%	16 = 33%	45 = 47%	85 = 26%
Total homologous "sensitized" knees involved (1 per animal)	0	3 = 43%	1 = 25%	6 = 75%	10 = 37%
Total non-homologous "sensitized" knees involved (1 per animal)	0	2 = 28%	1 = 25%	3 = 37%	6 = 22%
Total No. of joints involved, excluding "homologous sensitized" knees	4 = 4%	17 = 22%	15 = 34%	39 = 44%	75 = 25%

compared with those injected with the heterologous strain; but Streptococcus A135 in the amount inoculated had such high invasive powers that practically half the joints of the rabbits were inflamed. It therefore hardly seems justifiable to attribute the involvement of the injected knees to a specific sensitization.

In considering the total of all the experiments the knees injected with homologous strains showed only a slightly higher percentage of involvement than did other joints, and this can be accounted for largely by the figures in Experiment 3. If a definite hypersensitiveness of the joints had been induced by the experimental conditions a much higher proportion of "specifically sensitized" joints should have been inflamed.

It is impossible to coordinate our results with the conclusions of the workers previously quoted. As already mentioned, our analysis of Faber's experiments leads us to different conclusions from those that he drew; we feel, therefore, that his experiments and ours are more or less in accord. Herry states that he inoculated rabbits intravenously 8 to 15 days following intraarticular injection. Our measurements and observations of the rabbits' knees indicate that the inflammation incited by the intraarticular injection often had not completely subsided by the 12th to 15th day. It is not difficult to understand how rabbits with acutely inflamed knees, when inoculated intravenously, might have an increase of the inflammation in these joints. We cannot agree, however, that these streptococcus extracts are markedly toxic, or that intraarticular injection of them so alters the tissues of the joints that streptococci injected intravenously localize only in the treated joints.

Our experiments have nothing to do with the relation of an arthritis to a general allergic state. It is conceivable that an animal which is hypersensitive to a foreign protein or allergic to a certain bacterium, such as the tubercle bacillus, might have a severe arthritis if the foreign protein, specific bacillus, or bacterial protein were introduced into the joint. The arthritis in this event would be of the same nature as a local inflammation caused by the introduction of the specific antigen into the conjunctival sac or into the subcutaneous tissue. A discussion of a possible relation between this type of reaction and the manifestations of rheumatic fever is beyond the bounds of this article.

CONCLUSIONS.

It was impossible to demonstrate a condition of specific joint sensitization to non-hemolytic streptococci by first injecting the joints of rabbits with small doses of killed non-hemolytic streptococci, or with extracts of these organisms, and subsequently inoculating the rabbits intravenously with homologous living bacteria. Joints so treated were no more liable to involvement than were other untreated joints of the same animals.

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