THE INFLUENCE OF ALLERGY ON THE DEVELOPMENT OF EARLY TUBERCULOUS LESIONS *

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The influence of bacterial allergy in the form of tuberculin hypersensitiveness in accelerating the development of tubercle formation has long been generally recognized, and certain of its effects such as the intensification of inflammatory responses and the production of necrosis have been repeatedly emphasized. That allergy might play an even more fundamental rôle in the pathogenesis of the disease by determining, in part at least, the character of the anatomical lesions of tuberculosis has received little consideration. Yet, as the authors have pointed out in a previous paper,¹ the similarity of the histological reactions in most of the so-called infectious granulomas — syphilis, typhoid, glanders, brucellosis, tularemia and lymphogranuloma inguinale, for example — is so great that most pathologists candidly admit the dangers of confusion in routine diagnostic work. In all diseases of this group focal tissue reactions characterized by a predominance of large mononuclear phagocytes are the rule and in this same group of diseases bacterial allergy — defined as the first stage of the immune response to parenteral antigen, characteristic only of active as contrasted with passive immunity — is most regularly and persistently present and most highly developed.

Such a constant association of a morphological picture with an immunological state inevitably suggests the possibility of a causal relationship, though a more complicated interdependency cannot be excluded. If the relationship is a simple one, our alternatives are either that the presence of granulomatous lesions predispose to the development of a particularly high degree of bacterial allergy, or that bacterial allergy determines a focal mononuclear type of cytological response on the part of the host. That the latter actually occurs was the conclusion drawn by the authors in the work already cited and since confirmed in all essential details by Laporte.² Rössle,³ too, was evidently thinking along closely similar lines when he recently stated that granulomatous reactions can

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occur only in diseases in which allergy develops. For a more complete discussion of the relationship of allergy, not only to cytological responses but to the production of antibodies, the reader is referred to the review recently published by one of the authors.⁴

So far as any theory of the histogenesis of the tubercle may be said to have received general acceptance, the tubercle is usually looked upon as analogous to a foreign body reaction to a parasite which is relatively indestructible and possesses a high lipid content. Yet quantitative considerations of the amount of lipid in a few hundred tubercle bacilli, compared to the amount of tubercular or other lipid required to produce a noticeable foreign body reaction, obviously render such a hypothesis inadequate. Moreover, such an explanation could never even be promulgated to explain the nearly identical reactions to such different parasitic antigens as Gram-negative bacilli, spirochetes and filterable viruses.

The present work has, therefore, been undertaken to test further the applicability to tuberculosis of the hypothesis that allergy is the primary factor in determining the character of the early histological response to the infection in susceptible animals. If such a theory is tenable, it must be demonstrable (1) that the granulomatous lesions are not essential to the development of the delayed type of allergy, and (2) that tuberculin sensitivity develops at least as early as the appearance of the characteristic histological picture in the initial tuberculous lesion. The first of these points was demonstrated by Dienes⁵ some years ago in the following manner. Guinea pigs were injected intratesticularly with a large dose of tubercle bacilli. Twenty-four hours later, when control histological examinations showed simple abscess formation with a practically pure polymorphonuclear response and without a trace of the granulomatous reaction considered characteristic of spontaneous or experimental tuberculosis, a simple protein such as egg albumin was injected into the testicle. After a second 24 hour period the testicle, still free from any trace of granulomatous reaction, was excised. The animals, however, proceeded to develop after 3 to 4 days a high grade of delayed hypersensitivity of the tuberculin type to the injected egg albumin. Furthermore, in a later work Dienes and Mallory¹ showed that typical, albeit rather feeble, delayed sensitivity to non-bacterial proteins could be regularly produced in uninfected animals. The present experiments are, therefore, devoted to testing the second of the imposed conditions

by an analysis of the time factors governing the development of hypersensitiveness and the appearance of mononuclear infiltration, fibroblastic proliferation and other factors of the granulomatous tissue response.

EXPERIMENTAL

Methods

The general plan of the experiments was as follows: Guinea pigs were heavily infected with large doses of tubercle bacilli to ensure rapid development of detectable tuberculin sensitivity. Pairs of animals were later reinfected with small doses of bacilli at suitably chosen times so that there were available for histological study reinfectious lesions produced from 1 to 8 days after the first infection and from 1 to 3 days old. The sites of inoculation and of reinfection were varied in different sets of experiments to control as far as possible the effect of varying tissue substrates, and lesions of the omentum, the skin, the loose tissues of the groin and of the testicle were studied. In a preliminary series of 10 animals the first infection was in the peritoneal cavity; in the 3 subsequent series, comprising in all 29 animals, the first infection was testicular. In the first 3 series of experiments the R₁ low-virulent strain of tubercle bacilli was used in 10 mg. doses for the primary infection, and in 0.1 to 2 gm. doses for the reinfectious lesions. In the final set of 9 animals 5 mg. of a freshly isolated, virulent human strain was employed for infection, and 0.1 mg. of the same strain for reinfection. All animals were skin tested with 0.01 cc. of an unheated synthetic medium tuberculin which gave no reaction in uninfected pigs to determine the first appearance of tuberculin sensitivity. At appropriate intervals the animals were killed, the lesions — primary infections, reinfections and tuberculin reactions — excised, fixed in Zenker's fluid, stained with hematoxylin and eosin or eosin-methylene blue, and with Ziehl-Neelsen's stain. Serial sections were not made but 3 sections at different levels from each block usually sufficed to give satisfactory pictures of the lesions. Since the relative distribution of granulocytes and mononuclear phagocytes characteristically showed a reciprocal relationship in proportion to the distance from the center of the lesion, care was exercised to use for study only sections that could be shown to pass close to the center of the lesion.

Experiments

In the preliminary series 20 mg. of the R₁ strain was injected intraperitoneally and at the same time a dose of 2 mg. was given intracutaneously. Pigs were killed on the 2nd, 3rd, 4th, 6th, 7th and 9th days. Each pig had been injected intracutaneously with a small dose varying from 2 to 0.1 mg. R₁ bacilli the day before death. The peritoneal, the original intracutaneous and the 24 hour cutaneous lesions were all examined grossly and histologically. From the gross point of view the peritoneal lesions developed, as was expected, primarily in the omentum, though in a few animals lesions were seen in the mesentery or the tunica as well. Up to and through the 3rd day the omentum, though rolled up into a ball, swollen and hyperemic, could be fairly easily untwisted by gentle traction, but by the 4th day isolated firm lesions became evident and on subsequent days dense fibrous adhesions bound it into a solid tumor-like mass. Microscopically by the 3rd day mononuclear cells predominated and from the 4th day onward fibroblastic proliferation was evident, which rapidly resulted in the development of abundant granulation tissue.

In each of the guinea pigs killed from the 3rd day onward two skin lesions were available for study, one made simultaneously with the intraperitoneal injection, the second 24 hours before the pig was sacrificed. In the 3 day pigs both the 72 and the 24 hours old reactions were similar in character though materially different in extent. The reaction in each consisted essentially of polymorphonuclear infiltration with very few mononuclears and no evidence of fibroblastic proliferation. Reactions from the 4th day on — the 24 hour as well as the older reaction — were entirely different in character. Polymorphonuclear leukocytes were still abundant and tended to be clustered fairly compactly about the bacilli, but the surrounding tissue showed an extensive reaction with many mononuclear phagocytes hovering about the edges of the lesions and clumped about the blood vessels and nerve sheaths.

This preliminary set of animals served to emphasize the significance of the period from the 3rd to the 5th day, and in the subsequent series attention was especially concentrated on this period.

In several of these animals an interesting phenomenon characterized by a fiery red, hemorrhagic reaction of the parietal peri-

toneum developed. A similar local hemorrhagic reaction was noted in some of the animals described below in the group recorded as Series III, which were infected in the loose tissues of the groin.

A brief description of this phenomenon seems justified since we are unaware of any published report of a similar phenomenon in uncomplicated tuberculous infections, though Dienes⁶ has reported a similar lesion following the injection of egg white in tuberculous animals previously infected and sensitized by the intraperitoneal route. It was observed in about half of the intraperitoneally infected animals, which were sacrificed on the 5th, 6th and 7th days after infection, and in one instance was observed by laparotomy under local anesthesia to rule out agonal phenomena. The reaction consisted of multiple small hemorrhages, sometimes nearly confluent, in the parietal peritoneum, which ordinarily shows little or no gross change at this stage of intraperitoneal infection in contrast with the omentum which is heavily beset with lesions. It was particularly evident where bits of omentum or of exudate had become adherent to the peritoneum. Microscopically these areas showed in addition to the hemorrhage intense monocyctic infiltration, but no polymorphonuclear accumulation such as invariably is associated with the presence of the organisms themselves (Fig. 12).

In the guinea pigs of Series III a 5 mg. primary dose was given simultaneously in the groin and in the testicle. In the latter no hemorrhagic phenomenon appeared, but in the former 3 days after infection a zone of hemorrhage 30 mm. in diameter developed, which reached its maximum on the 4th day and then slowly disappeared during the next 4 days. A similar reaction has been noted in rabbits infected in the subcutaneous tissues.

Both the peritoneal and the subcutaneous lesions resemble closely both grossly and microscopically the flaring up of infection sites at the moment when allergy develops, and also the similar reactions recently reported from this laboratory following injection of human serum and of turtle egg in guinea pigs.⁷

It seems evident that these reactions are allergic in character and it is suggested that they indicate diffusion for some distance from the lesions through the tissues of tuberculin-active substances. This in turn renders possible the early sensitization of tissues in the immediate neighborhood of a lesion before generalized sensitivity develops.

Such features of the subsequent 3 series of experiments as are susceptible of tabulation are presented in Table I. From this it will be evident that no animal proved tuberculin-sensitive on the 2nd day, and no animal failed to show definite sensitivity on the 4th day. The reactions on the 3rd day appeared negative or equivocal on gross examination. When examined microscopically, using the criterion of significant mononuclear infiltration as evidence of a positive reaction, 3 out of the 7 examined microscopically were considered weakly positive.

The histological preparations of the tuberculin reactions showed the picture that we have previously described¹; a very slight and presumably non-specific reaction to the injected fluid characterized by a small number of scattered polymorphonuclear leukocytes in the 1st and 2nd day, and most of the 3rd day animals; marked constant mononuclear infiltration in all animals tested from the 4th day onward, with some tendency to the reappearance of polymorphonuclears as the degree of sensitiveness became more intense and necrosis appeared in the tissues. In a recent paper which essentially confirms our observations Laporte² describes edema and polymorphonuclear infiltration as the first stage of the histological process in a tuberculin reaction. At a later point in the paper he describes a similar result from injecting tuberculin into normal animals. Our findings are entirely in accord with his but we prefer to regard this reaction as a non-specific response to the trauma of the injection and to the faintly irritating effect of all tuberculin preparations rather than as an integral part of the tuberculin reaction.

In the reinfectious lesions (*i.e.*, intracutaneous or intratesticular injections of living tubercle bacilli in a previously infected pig) the invariable initial response was a flood of polymorphonuclear leukocytes which quickly clustered about the organisms with the formation of small abscesses. When the reinfection was produced 2 days after the primary lesion nothing more was noted, but in some of the 3 day examples, and constantly from the 4th day on, a noticeable alteration in the reaction appeared. There was greater hyperemia, the fibroblasts of the surrounding tissues appeared swollen and their nuclei hyperchromatic; in the early stages an occasional mitotic figure, and in the later stages numerous mitoses were found. Simultaneously, as far as we could judge, large num-

TABLE I

Day	Series I												Series II												Series III											
	Primary infection — 10 mg. R ₁ intratesticular Reinfection — 0.1 mg. R ₁ intracutaneous												Primary infection — 10 mg. R ₁ intratesticular Reinfections — 0.1 mg. intracutaneous												Primary infection — 5 mg. virulent TB, testicle or groin Reinfections — 0.05 mg. virulent TB, skin and testicle											
	Primary lesion			Tuberculin			R ₁ intracutaneous			Primary lesion			Tuberculin			R ₁ intracutaneous			Primary lesion			Tuberculin			Virulent Tb. intracutaneous											
	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross	P	M	Gross						
2	+++	±	3 x 4	±	±	3 x 4	+	o	Trace	±	o	o	+	o	o	+	+	±	+++	o	o	+	+	+	+	+	+	+	±	±						
	++	±	5 x 6	±	±	5 x 6	+	o	Trace	±	o	o	+	o	o	+	+	±	+++	±	o	Trace	+	+	+	+	+	+	+	o						
3	+++	+++	3 x 4 5 x 6	±	+	5 x 6 5 x 6	+++	+	Trace Trace	±	±	±	+	±	±	+	+	±	+++	+	o	Trace	+	+	+	+	+	+	+	o						
4							+++	+++	7 x 7	±	+	10 x 8	+	+	+	+	+	±	+++	+	+	8 x 7							+	+						
							+++	+++	7 x 6	±	+	9 x 8	+	+	+	+	+	±	+++	+	+	8 x 8							+	+						
5	++	+++	14 x 13	+	+++	12 x 10	++	+++	13 x 13	±	+++	14 x 13	+++	+	+	+	+	±	+++	+	+	15 x 13							+	+						
	++	+++	11 x 10	+	+++	10 x 10	++	+++	8 x 9	±	+++	13 x 11	+++	+	+	+	+	±	+++	+	+	15 x 13							+	+						
6	++	+++	13 x 12	+	+++	10 x 8	++	+++																												
	++	+++	13 x 11	+	+++	12 x 11	++	+++																												
8	++	+++	20 x 20	+	+++	15 x 10	++	+++																												
	++	+++	20 x 18	+	+++	16 x 15	++	+++																												

The letters P and M heading the columns stand respectively for polymorphonuclear leukocytes and large mononuclear phagocytes.
The proportions of these types of infiltrating cells have been roughly estimated by four grades from ± to +++.
The figures in the columns labelled "Gross" indicate the size of the discolored area in the skin in millimeters.

bers of mononuclear phagocytes appeared, first in the surrounding tissues at some distance from the organisms and polymorphonuclear leukocytes, but fairly quickly condensing in a layer several cells thick just outside the neutrophils to form a loosely encapsulating wall. This process first became manifest on the 3rd day in a few only of the animals. By the 4th day, when hypersensitivity is well marked, it was never lacking. Figure 1 illustrates the localized abscess which regularly develops when living tubercle bacilli in doses from 0.1 to 2 mg. are injected intracutaneously into either sensitized or non-sensitized animals. This particular one was produced in a non-sensitized animal with a dose of 2 mg. and was excised on the 3rd day. Sensitization as judged by the tuberculin test had not yet developed. Figure 2 is a higher power of the corium surrounding the abscess and shows that even on the 3rd day the cellular reaction in this instance was almost purely polymorphonuclear. In contrast, Figures 3 and 4, likewise from the corium just outside the central abscess, but from a 24 hour old reaction in a pig sensitized 4 days before the intracutaneous reinfection, show very few polymorphonuclears, marked monocytic infiltration and definite proliferation of fibrous tissue. Reinfected lesions in the testicle developed in an essentially similar manner to those of the skin. Such lesions were produced in the guinea pigs recorded as Series II in the tabulated summary by the injection of 0.2 mg. of R_1 bacilli in the opposite testicle from that used for the primary infection. Figure 5 shows the exudate at 24 hours in a testicle that was infected 2 days after the first infection. The exudate consists almost wholly of infiltrated polymorphonuclears and mononuclear cells are entirely lacking. Figure 6, made again from a 24 hour lesion in a testicle but one where reinfection occurred 4 days after the first infection, shows large areas of tissue densely infiltrated with mononuclear leukocytes.

A comparison of reinfected lesions with primary infections, when interpreted in the light of our previous studies of the mononuclear character of the cellular response to tuberculin hypersensitivity, clearly showed a change in the character of the reaction on the 3rd day. At this time the reaction to tuberculin was not usually clearly positive. Probably the non-resorbability of the bacilli themselves makes them a more sensitive reagent for determining the first trace of hypersensitivity than tuberculin.

THE PRIMARY INFECTION

The development of the lesions at the sites of the massive primary infections in the testicles, and for that matter in the soft tissues of the groin in Series II, proved remarkably uniform. During the relatively short periods over which the animals were observed, no noticeable difference was seen between the lesions produced by the R₁ and the virulent strain. After 24 hours the bacilli were found enclosed in an abundant exudate consisting entirely of polymorphonuclear leukocytes (Fig. 7), while the surrounding tissue showed marked hyperemia and edema. At 48 hours distinct changes could be seen in the fibroblasts; they appeared much larger and more prominent, the nuclei stained more intensely, and the cytoplasm had increased in volume. By 72 hours distinct progression of the lesion had occurred (Figs. 8 and 11). Mononuclear phagocytes were appearing in abundance, the connective tissue cells had clearly multiplied, and occasional mitotic figures were encountered. By the 4th day a thick wall of mononuclears and young fibroblasts surrounded and encapsulated the abscesses. Mononuclears began to penetrate the abscess and to replace the polymorphonuclears. By the 5th day the granulation tissue had extended far from the site of injection and formed wide bands between the atrophying parenchymal tubules (Fig. 9). The lesions continued to advance until the 8th day, the last one studied, with progressive replacement of the abscesses with tuberculous granulation tissue. Only on the last 2 days could the development of "epithelioid cells" from the mononuclears be made out.

Minor variations in this pattern were observed. In Series II, for instance, there was a distinctly greater connective tissue reaction on the 2nd day than in any of the other sets of animals. In the group where one of the primary sites of infection was the groin, an area of hemorrhage 30 mm. in diameter appeared on the 3rd day. This did not, however, appear to modify the usual progress toward encapsulation of the lesion.

SUMMARY OF EXPERIMENTAL EVIDENCE

The experimental evidence gathered from study of the tuberculin reactions and the reinfections may be summarized as follows: At a point not later than the 4th day, sometimes even on the 3rd

day, the reaction of the animals to tuberculin — usually more evidently to tubercle bacilli themselves — is clearly different in character from that observed in uninfected animals or in the first 2 days after the primary infection. It is quicker, far more intense in proportion to the dosage used, and microscopically can be shown to have the predominantly mononuclear character previously described¹ as characteristic of the delayed type of hypersensitivity. Even at this early stage the animals are clearly developing generalized tuberculin sensitivity.

At approximately the same time period — regularly on the 4th day, usually in incipient form on the 3rd day — a distinct change in the character of the primary lesion can be demonstrated. Whereas the reaction of the first 24 to 48 hours shows only congestion and serous exudation with marked accumulation of polymorphonuclear leukocytes — a reaction indistinguishable from that which might be produced by any pyogenic organism — beginning sometimes on the 3rd day and becoming well marked on the 4th, a second type of reaction has become obvious. Collections of large mononuclear phagocytes begin to appear as cuffs about the blood vessels and nerves, to infiltrate the stroma first at some distance from organisms and leukocytes but gradually to condense about them to form a wall several cells thick. At about this time the connective tissue cells begin to appear swollen and hyperchromatic, and mitotic figures begin to appear in abundance in them. In another 24 hours collagen begins to be laid down and true encapsulation is initiated. The parallelism between the appearance of demonstrable hypersensitivity and the alteration of the character of primary lesion are closer when both inoculations are made into similar tissues. When one injection is made into the peritoneal cavity and the other into the skin, for instance, noticeable differences in the rate of development of the lesions are apparent. The complications introduced by the absorption of large amounts of fluid exudate might readily explain the discrepancies however.

DISCUSSION

Under the conditions of the experiments that have been described it has been shown that a very close parallelism exists between the appearance of demonstrable tuberculin sensitiveness and the shift from a purulent to a granulomatous infiltration of the

tissues. More exact determination of the time factors seems scarcely feasible when one reflects that a faint skin reaction of the delayed type can only be read 24 hours after it is performed — an interval during which it is fair to assume that the degree of hypersensitivity has been progressively and rapidly increasing. The discrepancies observed, therefore, seem to fall within the limits of error of the method and it is not unreasonable to conclude that hypersensitivity and alteration in the character of the histological response — an alteration which for the first time begins to suggest the typical granulomatous lesion — appear simultaneously.

It is realized, of course, that the massive infections used in these experiments have no counterpart in spontaneous tuberculosis of man or beast, but purposeful exaggeration of natural phenomena is one of the most useful methods of studying them and has been used by choice or necessity in most of the experimental work on the disease. A negative result from our experiments would have been strong evidence against the validity of our thesis. The result obtained is of value as strongly suggestive confirmatory evidence, but does not of itself constitute proof of similar time relationships under conditions of minimal infection. With the usual “spontaneous” infection of man or animal it is certain that weeks, probable that months may pass before generalized tuberculin sensitivity develops.

Obviously, to maintain our thesis we must fall back on the theory of local hypersensitiveness, always a favorite theme of speculation but except in special instances tantalizingly difficult to demonstrate experimentally.

Certain observations of Stewart are of great interest in this regard. In a study⁸ of the histology of tuberculous infections of the testicle in guinea pigs rendered allergic with preliminary doses of heat-killed tubercle bacilli he noticed that attempts at healing — judged primarily on the basis of fibroblastic proliferation — became evident several days before generalized hypersensitivity could be recognized by skin tests. A probable explanation for this seemed to him to be the local development of allergy before it became generally detectable. In a second investigation⁹ he was able to demonstrate this by the injection of tuberculin directly into the infected testis. A reaction characterized by extensive necrosis of the tubular cells, and an acute inflammatory reaction with hem-

orrhage and fibrin deposit appeared regularly when the injection was made on the 3rd day and became more marked on subsequent days. It was not until the 11th day that he observed positive reactions from tuberculin injections into the other uninfected testicle or in the skin. The time relationships of slight reactions on the 3rd day, well marked ones from the 4th day onward, are remarkably close to those we have demonstrated by a different technique.

Other forms of localized hypersensitivity, less directly applicable to tuberculosis but significant because of the more perfect control which is possible, are well known. The cutaneous drug-idiosyncracies, for instance, are known frequently to remain limited to the segment of skin originally exposed. Even protein sensitizations may remain localized to the treated area, as in the experiments of Redfern¹⁰ and of Simon and Rackemann.¹¹ Not only sensitization but immunization, as represented by the presence of antibodies, has been shown on occasion to develop locally or to remain localized. Smith, Orcutt and Little,¹² for instance, showed that after inoculation of *Br. abortus* in one quarter of a cow's udder the antibodies appeared in highest concentration in the infected quarter. Recently McMaster and Hudack¹³ observed that after injection of bacteria into the ear of mice agglutinin appeared first in the corresponding cervical lymph node where it could be detected for some time in higher concentration than in the blood serum or in extracts of other lymph nodes. In the light of our theory that bacterial allergy represents the first phase of the normal immune process, it is only reasonable to suppose that it also should appear locally earlier and in higher degree than general sensitivity.

It would be unwise to conclude without pointing out certain limitations to what we feel are justifiable deductions from our experiments. We are not attempting to maintain and do not believe it tenable that allergy is the only cause of mononuclear infiltration or of granulomatous reactions. The response to agar-agar or to lipid injections is too obvious evidence to the contrary. Nor would we wish to be understood as refusing to credit to the lipoids of the tubercle bacillus or to those developing in the course of caseation a rôle in the determination of the later stages of the cytology of the tubercle. The rôle of allergy in the early stages of tuberculosis lies in the greatly increased rapidity and dispropor-

tionate intensity with which the granulomatous response develops, a rôle that is certainly not without importance for the understanding of the disease.

SUMMARY

A group of experiments designed to study the correlation of the early histological response to tuberculous infection with the development of allergy has been described and illustrated. In guinea pigs with massive primary infections of the peritoneal cavity or testicles, reinfectious lesions were produced on successive days and the animals simultaneously skin tested for tuberculin sensitivity. After the lapse of varying periods of time the primary and reinfectious lesions and the tuberculin reactions were compared histologically. Under the conditions employed and within the limits of error of the method, it was found that the first appearance of significant numbers of large mononuclear cells and the development of detectable tuberculin sensitivity occurred simultaneously between the 3rd and 4th days after the primary infection. The significance of these observations in relation to the authors' previously advanced hypothesis that bacterial allergy is responsible for the early granulomatous reaction of the host has been discussed.

REFERENCES

1. Dienes, Louis, and Mallory, Tracy B. Histological studies of hypersensitive reactions. *Am. J. Path.*, 1932, 8, 689-708.
2. Laporte, R. Histo-cytologie des réactions locales d'hypersensibilité chez le cobaye (réactions allergiques à la tuberculine et réactions anaphylactiques). *Ann. Inst. Pasteur*, 1934, 53, 598-640.
3. Rössle, R. Die nosologische Stellung des Rheumatismus. *Klin. Wchnschr.*, 1936, 15, 809-814.
4. Dienes, L. The specific immunity response and the healing of infectious diseases. Significance of active immunity and the connections between the immunity response and the anatomic lesions. *Arch. Path.*, 1936, 21, 357-386.
5. Dienes, L. Ueber die Wirkung des tuberkulösen Krankheitsherdes auf die Immunitätsreaktionen des Organismus. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1930, 68, 13-42.
6. Dienes, L. Hemorrhagic reactions in tuberculous lesions and skin tests during protracted anaphylactic shock. *Proc. Soc. Exper. Biol. & Med.*, 1930, 27, 690-692.

7. Dienes, L., and Simon, F. A. The flaring up of injection sites in allergic guinea pigs. *J. Immunol.*, 1935, 28, 321-330.
8. Stewart, Fred W. Testicle infection in guinea pigs sensitized with killed tubercle bacilli. *J. Immunol.*, 1926, 11, 123-134.
9. Stewart, Fred W. Observations on focal tuberculin hypersensitiveness in an infected organ previous to a general sensitization. *Am. J. Path.*, 1925, 1, 495-501.
10. Redfern, William W. Skin reactions produced by antihuman serum. *J. Immunol.*, 1930, 18, 109-120.
11. Simon, Frank A., and Rackemann, Francis M. The development of hypersensitiveness in man. I. Following intradermal injection of the antigen. *J. Allergy*, 1934, 5, 439-450.
12. Smith, Theobald, Orcutt, Marion L., and Little, Ralph B. The source of agglutinins in the milk of cows. *J. Exper. Med.*, 1923, 37, 153-174.
13. McMaster, Philip D., and Hudack, Stephen S. The formation of agglutinins within lymph nodes. *J. Exper. Med.*, 1935, 61, 783-805.

DESCRIPTION OF PLATES

PLATE 133

- FIG. 1. The abscess produced by the intracutaneous injection of 2 mg. of R₁ tubercle bacilli in a previously normal guinea pig. It was excised on the 3rd day after infection. $\times 20$.
- FIG. 2. A field from the corium at a little distance from the central abscess in this same case. The inflammatory response still consists entirely of polymorphonuclear leukocytes. $\times 100$.
- FIG. 3. A field from the corium bordering the central abscess produced by the intracutaneous injection of 0.1 mg. of R₁ tubercle bacilli. The guinea pig had been primarily infected 4 days earlier with 10 mg. of R₁ bacilli in the testicle, and the skin lesion was excised at the end of 24 hours. It shows rare polymorphonuclears, numerous monocytes and an increase in fibroblasts. $\times 100$.
- FIG. 4. The same. $\times 300$.
- FIG. 5. Exudate in the testicle some distance from the central abscess 24 hours after the injection of 0.1 mg. R₁ bacilli in a guinea pig primarily infected 2 days before with 10 mg. of tubercle bacilli in the opposite testicle. A simultaneous tuberculin test was negative. The great predominance of polymorphonuclears and the paucity of mononuclears are evident. $\times 250$.
- FIG. 6. A corresponding field from a 24 hour old testicular lesion of a slightly allergic animal in which the reinfectious lesion was produced 4 days after the primary infection. The reaction at this spot is almost wholly mononuclear. $\times 250$.

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PLATE 134

FIGS. 7, 8, 9 and 10 show primary lesions of the testicle produced by infection with 5 mg. of R_1 tubercle bacilli. They are respectively 1, 3, 5 and 8 days old.

Fig. 7. At 1 day a poorly defined abscess only is apparent.

Fig. 8. At 3 days a narrow rim of mononuclear infiltration and granulation tissue is evident.

Fig. 9. At 5 days the granulomatous encapsulation is broad and extends in narrow bands out into the surrounding parenchyma.

Fig. 10. At 8 days the abscess has been completely replaced by the granulomatous mass which occupies nearly the entire testicle. $\times 6$.

FIG. 11. A portion of the reacting tissue outside the abscess from the same testicle as Fig. 8, a 3 day lesion. In this area the reaction consists almost solely of mononuclear phagocytes. $\times 250$.

FIG. 12. The peritoneum of an intraperitoneally infected pig which shows the hemorrhagic reactions described in the text. In this area marked mononuclear infiltration is present. $\times 100$.

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