THE RÔLE OF HYPERSENSITIVITY IN THE PRODUCTION OF EXPERIMENTAL MENINGITIS

I. Experimental Meningitis in Tuberculous Animals

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The hypersensitive state may be of significance in the pathogenesis of certain central nervous system disturbances. Of primary importance is the possible relationship of the postexanthematous and postvaccinal encephalitides to an allergic state of the central nervous system. Glanzmann (1) and Rivers (2) emphasize the hypersensitive state as a rôle in the nervous complications of chicken-pox, smallpox, measles and vaccination. Rackemann (3) suggests that certain of the migrains may be due to the patient's idiosyncrasy to some foreign protein. A review of cases of epileptics, whose attacks were relieved when food and other products to which they were found hypersensitive were removed from their diets and environment respectively, will be found in the monograph on epilepsy by Lennox and Cobb (4). The relationship between allergy and the severity of tuberculous meningitis will be referred to later. It was because of the suggested association of allergy to the above mentioned nervous maladies that the following experiments were designed. We wished to study the potentialities of the central nervous system in sensitized animals to react when brought in contact with the homologous antigen.

Tuberculous animals were first employed because it is known that their tissues are sensitive to tuberculin. The subarachnoid space was chosen as the locus of study because of its easy accessibility through the basal cistern and, furthermore, because of its very intimate relationship with the brain parenchyma. The direct extension of the subarachnoid space around the blood vessels into the depth of the brain parenchyma to envelop the individual nerve cells in all probability, is

known from the work of Weed (5) and Cushing (6). It is impossible, in our judgment, to separate the subarachnoid space as a space in which spinal fluid bathes the surface of the brain from its extension to the very nerve elements of the brain proper. Such being the case, any reaction which can be made to occur in the fluid spaces covering the brain surface, will, in all probability, result likewise in the deeper portions of the brain substance under proper experimental conditions.

Methods

Guinea pigs averaging 200 to 300 gm. were inoculated subcutaneously in the groin with a light suspension of freshly isolated virulent human tubercle bacilli. The tubercle bacilli were grown, either on glycerine agar slants or Dorset's egg media. The tuberculosis in the animals was permitted to progress to various stages before the test inoculation was made. Old tuberculin was diluted in physiological saline to the concentration desired. Not more than 0.2 to 0.3 cc. of each dilution could be safely inoculated into the basal cisterns of the animals. A small hypodermic needle, size No. 26, measuring $\frac{3}{8}$ inch in length was used. It was unnecessary to anesthetize the animals for the procedure. The immediate pressure symptoms that developed after the inoculation indicated that the injection mass had entered the cisterns. These symptoms disappeared within a few seconds with complete recovery. The point of inoculation into the basal cisterns was determined by finding the crest of the occipital bone at the midline and puncturing the skin 1 cm. below this point. The needle was then permitted to strike the base of the occipital bone in the midline and forced gently downward until the rim of the foramen magnum was reached. At this point the needle perforated the occipital-atlantoid ligament and the dura with ease. Extreme care was taken to see that the needle did not penetrate too far so as to cause trauma to the brain stem. The material was slowly injected. Observations were frequently made for any clinical symptoms that could be attributed to the traumatic effects of the material inoculated into the subarachnoid space. If there was no change observed, the animals were permitted to live for various lengths of time. The animals that did not die were killed (under complete ether anesthesia) by means of decapitation. They were immediately autopsied. Both gross and microscopic studies were made of the viscera in order to determine the extent of the tuberculosis. The brain was removed, sectioned and fixed immediately in 95 per cent alcohol and stained with iron-hematoxylin and eosin and Nissl techniques. Bacteriological methods were employed in aseptically removed portions of the brain to rule out presence of either the ordinary bacteria or the tubercle bacillus. The inoculation of tuberculin into the carotid arteries was performed under ether anesthesia.

Every experiment included controls of both the tuberculous and non-tuberculous animals. The tuberculous animals had glycerine broth equivalent to the concentration and dosage found in old tuberculin inoculated into the basal cistern. Also,

several animals with far advanced tuberculosis were sacrificed without having received any injection in the basal cistern. Histological sections were prepared as described above. Many non-tuberculous guinea pigs were likewise inoculated *via* basal cisterns with old tuberculin and glycerine broth.

RESULTS

Several preliminary experiments were devised in order to determine the nature of the clinical response and the type of lesion produced in the central nervous system by the inoculation of tuberculin into tuberculous animals. The results were constant both clinically and pathologically.

Within 4 to 5 hours after the inoculation of tuberculin into the cisterns, restlessness and ruffling of hair appeared which became progressively more severe until twitchings and convulsions resulted. Marked weakness, incontinence and opisthotonus were followed shortly by death which occurred within 6 to 12 hours. The tuberculous animals receiving glycerine broth as well as the non-tuberculous animals which received tuberculin never showed any clinical evidence of cerebral irritation.

When histological sections of the brains were prepared from tuberculous guinea pigs inoculated with tuberculin, there was found in the subarachnoid space, an extensive exudate which extended over the convexities of the brain and into the sulci. In many instances there was a subpial as well as a marked cortical perivascular infiltration. There was no demonstrable evidence of damage to the cells of the brain parenchyma. The control animals showed either a very slight non-specific reaction or none at all. Tuberculous animals that received the glycerine broth *via* the cisterns, always responded histologically by a very slight non-specific type of reaction.

After having established the response in a hypersensitive animal, primarily in the leptomeninges of the central nervous system, an experiment was devised to determine the period of maximum response and to correlate the clinical manifestations with the histological findings as shown in Table I. The animals used for this experiment had far advanced generalized tuberculosis. The quantity of tuberculin inoculated *via* basal cisterns was 0.3 cc. of a 1/100 dilution in physiological saline.

The results demonstrate that in guinea pigs showing extensive generalized tuberculosis there rapidly develop definite clinical symptoms that are apparently associated with the anatomical response of

the leptomeninges. Histological changes were not demonstrable until 4 hours after the inoculation of the tuberculin. The maximum response occurred within 8 to 12 hours. There were slight variations noted in the quantity of exudate in the meninges, but this may be attributed to the differences in response of the individual animal, as well as upon the variation of the amount of visceral tuberculosis present.

Since guinea pigs with far advanced generalized tuberculosis uniformly developed the most widespread exudate in the subarachnoid space within 8 to 12 hours, it was possible to take this time as a maximum period after the inoculation of tuberculin for sacrificing the animals. An experiment was devised to study the relation between

TABLE I

Total No. of animals	Survival time after tuberculin inoculation	Clinical symptoms	Lesions of the central nervous system
	hrs.		
4	2	None	No lesions
4	4	"	Definite polymorphonuclear exudate in sulci and over convexities of brain
4	6	Definite progressive weakness and twitchings	Extensive polymorphonuclear exudate over convexities with marked subpial and perivascular infiltration
8	8–12	Death of all animals	" "

the amount of visceral tuberculosis and the severity of the hypersensitive response in the leptomeninges.

A series of guinea pigs were inoculated subcutaneously into the groin with a virulent strain of human tubercle bacilli. At designated intervals (Table II) two guinea pigs were selected from the group and each inoculated with 0.3 cc. of a 1/100 dilution of tuberculin in physiological saline. If the animals did not develop symptoms and die before 12 hours, they were sacrificed. A skin sensitivity test was performed 24 hours before the inoculation of the tuberculin into the basal cistern. Bacteriological studies, both for the presence of tubercle bacillus and for aerobic and anaerobic bacteria were made with portions of the brain tissue.

Non-tuberculous animals inoculated in the basal cistern with tuberculin and tuberculous animals inoculated with glycerine broth were used as controls. Some

TABLE II

Interval between initial inoculation and tuberculin inoculation	Result of skin test	Clinicial symptoms	Extent of visceral tuberculosis	Lesion of central nervous system
days				
5	Neg.	None	No gross or microscopic evidence of tuberculosis	Slight but definite exudate at base of brain More than a nonspecific response
7	"	"	" "	" "
10	Pos.	Restlessness	Inguinal lymph nodes enlarged; few microscopic tubercles in spleen and lungs	Moderate amount of exudate in sulci and over convexities of brain with perivascular infiltration
12	"	Marked restlessness	Same as for 10 day period	Moderate amount of exudate in sulci and over convexities of brain with subpial infiltration
14	"	Marked restlessness with progressive weakness	Enlarged inguinal lymph nodes with necrosis; microscopic tubercles in spleen and liver	Extensive exudate in sulci and diffuse over convexities of brain with subpial and perivascular infiltration
19	"	Twitching of muscles, restlessness and progressive weakness. Loss of sphincter control	Inguinal lymph nodes and spleen enlarged and nodular; microscopic tubercles in lung and spleen	Extensive exudate in sulci and over convexities of brain with perivascular infiltration
24	"	Onset of twitching of muscles within 5 hrs. Convulsions and death within 6 hrs.	Inguinal lymph nodes enlarged and necrotic; gross evidence of tubercles in lungs and spleen with microscopic tubercles in liver	Extensive exudate in sulci and over convexities of brain; subpial and perivascular infiltration very distinct

TABLE II—*Concluded*

Interval between initial inoculation and tuberculin inoculation	Result of skin test	Clinical symptoms	Extent of visceral tuberculosis	Lesion of central nervous system
days				
25	—	Rapid onset of twitchings and convulsions resulting in death within 5½ hrs.	Inguinal lymph nodes enlarged and caseous; gross evidence of tubercles in lungs and spleen; microscopic tubercles in liver	Extensive exudate in sulci and over convexities of brain; marked subpial and perivascular infiltration
31	—	Rapid onset of weakness and convulsions. Loss of control of sphincters. Death within 9 hrs.	Gross evidence of tubercles in lungs, spleen and liver; inguinal lymph nodes are caseous	Extensive exudate in sulci and over convexities of brain
37	Pos.	Rapid onset of weakness and death within 6 hrs.	Extensive generalized tuberculosis throughout organs	Extensive exudate in sulci and over convexities of brain; marked subpial and perivascular infiltration

of the tuberculous animals were inoculated with tuberculin into the neck muscles, and others that received no injection of tuberculin were sacrificed from time to time. These controls never showed any clinical symptoms nor was there any evidence of anatomical changes in the subarachnoid spaces comparable to those found in the experimental animals. The tuberculous animals that received glycerine broth showed a slight non-specific response characterized by a polymorphonuclear exudate.

These results indicate that the degree of the response of the central nervous system tissues to tuberculin is directly related to the extent of the visceral tuberculous disease.

The following two experiments (Experiments III and IV) were devised to determine the effects of high dilutions of tuberculin upon the leptomeninges and to study the transformation in the exudate produced within the leptomeninges.

TABLE III

Duration of infection	No. of animal	Survival time after tuberculin inoculation	Clinical symptoms	Extent of visceral tuberculosis	Lesions of the central nervous system
wks.		*hrs.*			
1	1	D 24	None	Very slight inguinal lymph node enlargement. No microscopic evidence of tubercles	No exudate or change observed
	2	K 48	"		
	3	K 120	"		
	4	K 120	"		
3	5	K 24	Every animal showed slight restlessness, twitchings and ruffled hair	Definite enlargement of inguinal lymph nodes. Microscopic evidence of tubercles in liver, spleen and lungs	Slight exudate in sulci consisting of equal proportions of lymphocytic and polymorphonuclear cells
	6	K 48			
	7	D 96			No exudate
	8	K 120			Slight lymphocytic exudate in sulci and over convexities
5	9	D 8	Marked restlessness, twitchings and weakness	Inguinal lymph nodes enlarged; extensive tubercles throughout lungs, liver and spleen	Extensive exudate in sulci and over convexities; polymorphonuclear and few lymphocyte cells
	10	K 24	Marked restlessness, twitchings and ruffling of hair	Inguinal lymph node enlargement; tubercles in liver, spleen and lungs	No exudate in meninges
	11	D 5½	Progressive and rapid development of symptoms and death	Extensive generalized tuberculosis	No exudate
	12	D 5½	" "	" "	Extensive polymorphonuclear exudate in sulci and over convexities with perivascular infiltration

D = dead. K = killed.

The tuberculous animals were chosen at intervals after the initial infection and inoculated with 0.3 cc. of a 1/10,000 dilution of tuberculin in physiological saline. The animals surviving for 120 hours were then sacrificed.

In addition to the usual controls, two others were used. One animal at the 1 week period was inoculated with a 1/10 dilution of tuberculin. It had no clinical symptoms and very slight polymorphonuclear exudate at the base of the brain. The other animal was chosen at the 3 week period and also inoculated with a 1/10 dilution resulting in the death of the animal within 12 hours and a very extensive polymorphonuclear exudate in the leptomeninges.

The results of the experiment are definite as concerns the clinical findings and pathological picture.

As the tuberculous process advanced within the viscera of the guinea pigs, there was a rapidly increasing susceptibility of the leptomeninges to the tuberculin. The central nervous system showed a quantitatively less and qualitatively different type of reaction from the tuberculous guinea pigs inoculated with more concentrated dilutions of tuberculin. When the animals were killed or died within 24 to 48 hours, the exudate was limited chiefly to the base of the brain and Sylvian fissures. The exudate usually consisted of equal proportions of the polymorphonuclear and lymphocytic cells. If the guinea pigs were not sacrificed or did not die before 120 hours there was observed either a very slight amount of residual exudate composed chiefly of small lymphocytic cells or the brain showed no anatomical changes. An occasional guinea pig, however, especially in this experiment, would manifest definite clinical symptoms as intense and typical as those previously described, but upon examination of the sections from the brain, there was no exudate present or any other demonstrable lesion. This phenomenon was noticed twice in the experiments in which greater concentrations of tuberculin (1/10 and 1/100) were inoculated into the basal cisterns. These guinea pigs at the time of inoculation of the material into the basal cistern showed very definite immediate pressure symptoms with the usual rapid recovery which indicated that the material reached the basal cistern. A possible explanation is that a few guinea pigs from a group are refractory to reacting in an acute manner; in other words, a state of anergy is present.

The following experiment was likewise performed to produce an exudate within the subarachnoid space as well as to permit the animal to survive.

Guinea pigs were used that showed a stage of the tuberculosis in which the lymph nodes and spleen were definitely enlarged, but the other organs in the gross did not show the presence of tubercles; in other words, a tuberculous infection was established which had not become generalized. One of two dilutions of tuberculin (1/100 and 1/1000) was inoculated into the basal cistern. If the animals did not die at the time desired, they were sacrificed.

TABLE IV

Dilution of tuberculin	No. of animal	Survival time after tuberculin inoculation	Clinical symptoms	Extent of visceral tuberculosis	Lesions of the central nervous system
		hrs.			
1/100	1	K 12	Gradual weakness, restlessness and twitchings of muscles	Inguinal lymph nodes enlarged and caseous. Gross tubercles in spleen; microscopic tubercles in lungs	Moderate amount of polymorphonuclear exudate in sulci and over convexities. Some perivascular infiltration
	2	D 36	Few hrs. before death, developed twitching of muscles, paralysis of hind legs and weakness	" "	Extensive polymorphonuclear exudate and a few mononuclear cells in sulci and over convexities
	3	D 60	Few hrs. before death, developed twitchings and extreme weakness	" "	Moderate lymphocytic exudate at base of brain with slight amount over convexities
1/1000	4	D 6	Gradual loss of power in hind legs, weakness and death	Inguinal lymph nodes enlarged and caseous. Gross tubercles in lungs, liver and spleen	Extensive polymorphonuclear exudate with subpial and perivascular infiltration
	5	K 60	Ruffling of hair	Inguinal lymph nodes enlarged and caseous. Spleen and liver show gross tubercles. Microscopic tubercles in lungs	Moderate lymphocytic exudate at base of brain, and slight amount over convexities
	6	D 120	Gradual loss of weight, progressive weakness, death	Generalized tubercles throughout viscera	" "

D = dead. K = killed.

The results of the experiment show the variation noted in the previous experiments.

The animals that were killed or died within the 6 to 12 hour period, responded with an exudate that extended over the convexities of the brain as well as showing marked perivascular infiltration. The exudate consisted entirely of polymorphonuclear and large mononuclear cells. The guinea pigs that survived from 12 to 120 hours, showed an exudate which was limited chiefly at the base of the brain and appeared slightly over the convexities. But, instead of a polymorphonuclear cellular response such as was found consistently in the guinea pigs dying early, there was a lymphocytic cell present. The cause of death of the animals could not be correlated with the exudate found in the subarachnoid space. It is known that when an exudate, not associated with necrosis, resolves, there is a gradual decrease of polymorphonuclear cells of the exudate and there is to be seen a mononuclear type of cell, the lymphocyte. This in turn gradually disappears so that the organ is returned to its apparently initial state. Since there is no evidence of necrosis in this response of the meninges, the exudate found in the subarachnoid space of the animals that survived for several days was probably the result of a gradual resolution of the more acute process that was previously elicited.

Thus far all the experiments have been limited to the inoculation of material into the subarachnoid space, which brought about the vigorous response in the leptomeninges. The cells of the brain parenchyma showed no evidence of damage by the technique used for this study. In order to bring the tuberculin into closer contact with the ganglion cells of the cortex, the skull was trephined while the animal was under ether anesthesia, the needle inserted directly into the cortex and the material injected. A dilution of tuberculin 1/100 was inoculated. The results are presented in Table V.

Instead of damage to the surrounding cells of the brain, there developed extensive generalized meningitis with marked perivascular infiltration. All the animals died within 6 to 8 hours after the inoculation. This response was similar to the reaction obtained when the material was inoculated by the basal cistern.

Carotid injections of tuberculin were done in guinea pigs with far advanced tuberculosis. The results were uniformly negative in the few animals observed. This phase of the problem is being studied.

Controls of both tuberculous and non-tuberculous animals were employed for every experiment. A summary of the controls is given in Table VI.

The complete lack of clinical symptoms and the absence of path-

TABLE V

No. of animal	Clinical symptoms	Extent of visceral tuberculosis	Lesions in central nervous system
1	Restlessness, twitchings, weakness and death in 8 hrs.	Extensive generalized tuberculosis	Moderate polymorphonuclear exudate in sulci and over convexities with perivascular infiltration
2	Restlessness, twitchings, weakness and death in 6 hrs.	" "	Extensive polymorphonuclear exudate in sulci and over convexities with perivascular infiltration
3	" "	" "	Extensive polymorphonuclear exudate in sulci and over convexities. Hemorrhage along the needle tract
4	None	None	Very slight polymorphonuclear and lymphocytic exudate in sulci

TABLE VI

No. of animals	Type of material inoculated into cisterna	Quantity inoculated	Clinical symptoms	Lesions in viscera	Lesions of the central nervous system
		Tuberculous animals			
		cc.			
22	Glycerine broth	0.3	None	Extensive visceral tuberculosis	Very slight polymorphonuclear exudate
4	Tuberculin into muscles of neck and peritoneal injection	0.3	"	" "	No reaction
3	No inoculation	—	"	" "	" "
		Non-tuberculous animals			
12	Glycerine broth	0.3	None	None	No reaction
23	Tuberculin	0.3	"	"	" "
4	No inoculation	—	"	"	" "

ological changes in the central nervous system is in strong contrast with the findings in the hypersensitive animals. All of the control

tuberculous animals showed a very slight, non-specific, polymorphonuclear exudate in response to the glycerine broth inoculated into the basal cistern. None of the controls died from the inoculation.

Both living and dead tubercle bacilli were next tested to determine the response of the leptomeninges in hypersensitive animals.

In the case of living tubercle bacilli suspensions of two concentrations were used. One consisted of a concentration of tubercle bacilli that gave an opalescent appearance while the other suspension consisted of a concentration of tubercle bacilli

TABLE VII

No. of animals used	Clinical symptoms and survival period	Extent of tuberculosis in viscera	Lesions of the central nervous system
3	Rapid progressive symptoms of weakness, twitchings and convulsions. Death in 3 to 5 hrs.	Enlarged inguinal lymph node. Spleen enlarged and nodular. Few tubercles in liver and lungs	Extensive polymorphonuclear exudate in sulci and over convexities of brain with marked perivascular infiltration
1	Ruffling of hair, anorexia, weakness of hind legs, death within 10 days	Enlarged caseous inguinal lymph nodes. Liver and lungs show many tubercles	Exudative and proliferative meningitis. Tubercle formation in perivascular spaces and parenchyma. Thrombi in vessels of brain
		Controls	
4	No reaction. Killed at 24 hrs.	None	Very slight non-specific polymorphonuclear exudate deep in the sulci

that contained not over 250 bacilli per high power field. In Table VII the effects of the concentrated suspension of tubercle bacilli in allergic animals are shown.

The concentrated living tubercle bacilli were capable of eliciting a very vigorous response in the leptomeninges of a hypersensitive animal. The animal that survived for a period of 10 days showed an exudate and proliferative type of response. There were many tubercles throughout the perivascular spaces of the brain.

Table VIII demonstrates the results of inoculating minute quantities of living tubercle bacilli into the basal cistern of tuberculous animals.

The number of days that the animal survived after the inoculation of the organisms is noted.

In comparing the lesions in the central nervous system of the animals that have far advanced visceral tuberculosis with those of the non-tuberculous animals, there is observed the absence of tubercles

TABLE VIII

No. of animal	Survival period after cisterna inoculation	Extent of visceral tuberculosis	Lesions found in central nervous system
250 tubercle bacilli per high power field			
	days		
1	2	Extensive generalized tuberculosis	Slight amount of lymphocytic infiltration in meninges
2	9	Moderate generalized tuberculosis	Very slight lymphocytic response of the meninges
3	23	Extensive generalized tuberculosis	Slight proliferative and lymphocytic exudate in the meninges
4	27	" "	" "
5	29	" "	Moderate degree of proliferative and exudative response. Exudate predominantly lymphocytic. Occasional tubercle in cortex
6	29	" "	" "
Controls (non-tuberculous)			
7	30	Lymphoid hyperplasia of spleen	Extensive proliferative and exudative response. Many tubercles in meninges and throughout cortex
8	20	" "	" "
9	25	" "	" "
10	28	" "	" "
11	29	" "	" "
12	2	Dead—pneumonia	No evidence of change

in the former group of guinea pigs. The very slight lymphocytic response in the leptomeninges is the only evidence of change in the central nervous system, while the control animals revealed marked evidence of proliferation and tubercle formation throughout the cortex and meninges. Even the tuberculous animals that lived as long as the controls showed only an occasional tubercle in the brain. Soper and

Dworski (7) have clearly demonstrated in their experiments of superinfection in the meninges of rabbits this difference between tuberculous and non-tuberculous animals. Dead tubercle bacilli were studied as to the type of response that would be elicited in the hypersensitive animal. A portion of the same suspension prepared for the experiment used in studying the effects of large concentrations of whole tubercle bacilli was heated in a water bath at 60°C. for 1 hour. The viability of the culture was tested by inoculation of the heated

TABLE IX

No. of animals used	Clinical symptoms and survival period	Extent of visceral tuberculosis	Lesions of central nervous system
3	Ruffling of hair, twitchings, convulsions and death within 4 to 12 hrs.	Extensive generalized tuberculosis throughout the viscera	Extensive polymorphonuclear exudate in sulci and over convexities of brain. Marked subpial and perivascular infiltration
	Controls		
2	No clinical symptoms. One animal sacrificed within 12 hrs.; the other permitted to live for 48 hrs.	None	Slight polymorphonuclear exudate at the base of the brain
1	Not inoculated	Extensive generalized tuberculosis	No exudate or change in the central nervous system

culture into normal guinea pigs. There was no evidence of tuberculosis 2 months after the initial inoculation.

Dead tubercle bacilli are capable of eliciting a response of the meninges in allergic animals.

DISCUSSION

In comparing the response of the leptomeninges in these experiments with the allergic response in other organs of tuberculous animals our results are in general those one might expect to find. The sterile meningitis produced in animals allergic to tuberculin is similar in its fundamentals to the tuberculin reaction of the skin in hypersensitive

animals. The seat of the response lies in the tissue spaces of the leptomeninges instead of the subcutaneous tissues. Similar allergic states have been produced in other organs of the tuberculous animals. Austrian and Willis (8), Soper and coworkers (9) and more recently Larson and Long (10) have produced pneumonia in tuberculous animals by bringing tuberculin in contact with the lungs. Long and Finner (11) and Long and coworkers (12) produced an acute glomerular nephritis in tuberculous animals by injecting tuberculin in particulate state into the renal artery. Long (13) has demonstrated marked changes in the testis of tuberculous animals due to tuberculin inoculations.

No apparent differences exist between the response that occurs in the lungs, kidneys and testis and the one that occurs in the meninges. The response is characterized by an exudate that is composed chiefly of polymorphonuclear cells. It is elicited within 6 to 12 hours after the inoculation of the tuberculin. The death of the animal may result, depending upon the location of this response. If the animal survives, the recovery takes place by resolution or by organization, depending upon whether there is necrosis present. Necrosis in the meninges, however, was not common. The resolving exudate consists chiefly of small lymphocytic cells.

When tuberculin was inoculated directly into the carotid artery of allergic animals there was no response elicited in the meninges of the hypersensitive animals. This lack of response is attributed to the failure of the antigen to remain localized for sufficient length of time and also to the dilution of the tuberculin. An attempt to demonstrate the presence of tuberculin in the spinal fluid of one of these animals failed. This failure may be due to technique; further attempts to demonstrate it are necessary. If sufficiently large quantities of tuberculin can be given without bringing about the death of the animal, it may be possible to establish the response in the central nervous system. Or, if the tuberculin is permitted to remain localized within the brain substance by means of embolic particulate matter, the allergic response will undoubtedly result.

Unequal intensity and rapidity of response of the organs when the body as a whole is sensitized to a foreign protein is a possibility. Stewart (14) found that the tuberculin reaction became positive on the

5th day in the testicle which was the primary focus of infection, and did not appear in the opposite organ until later. The leptomeninges of the tuberculous guinea pig responded within 5 days by a definite specific reaction, while the skin manifestations did not appear before the 10th day. However, the meninges were not the focus of primary infection. It is possible that the early response of the meninges is due to the anatomical structure of the organ and exudate, being more readily demonstrable in the earliest stage.

Glycerine broth always elicited a non-specific exudative response in the leptomeninges of tuberculous animals, while an equivalent quantity of glycerine broth administered to non-tuberculous animals yielded a very slight exudative response or none. Friedberger and Gajzago (15), Borrel (16), Somerfeld and Ziskin (17) found that tuberculous animals responded more vigorously to non-specific proteins than did the non-tuberculous animals.

Both dead and living tubercle bacilli when inoculated in sufficient quantities *via* the basal cistern of tuberculous animals caused an acute and vigorous response which terminated in the death of the animal, while smaller quantities of living tubercle bacilli resulted in a less severe acute reaction and a greater portion of the animals survived for a longer period of time. Histological sections of the brains obtained from the tuberculous animals that had living tubercle bacilli inoculated into the basal cisterns showed only an occasional tubercle and a moderate number of small lymphocytes at the base of the brain. On the other hand, the non-tuberculous animals that received small quantities of living tubercle bacilli had an extensive proliferative and exudative tuberculous response throughout the meninges and brain substance. Soper (7) has shown that the introduction of tubercle bacilli into an already tuberculous subarachnoid space will produce a sudden exacerbation of meningeal signs in the animals. There is an associated increase in cell count of the spinal fluid. The primary inoculations of non-tuberculous animals *via* the leptomeninges does not result in an acute onset of meningeal signs, but leads to a slow progressive type of tuberculous meningitis. He demonstrated that the superinfected animals did not reveal so extensive a type of meningitis when compared with the control animals. The primary focus in Soper's experiments was in the central nervous system, while the

primary focus in our animals was established in the viscera by inoculation into the groin. Both Austrian (18) and Borrel (16) found that the injection of tuberculin into the subarachnoid spaces of an animal with tuberculous meningitis during the latent period aggravated the symptoms and produced a more rapidly fatal termination of the animal's life.

Clinical observations of tuberculous meningitis have been reported in the literature in which the injection of tuberculin into the subarachnoid spaces has resulted in a definite acute exacerbation of the meningeal symptoms. Grace-Colvert (19) claims that the use of tuberculin in the subarachnoid space as a therapeutic measure in tuberculous meningitis may light up a solitary tubercle and give rise secondarily to a tuberculous meningitis. The acute symptoms described are undoubtedly due to the tuberculin acting on the allergic tissues of the leptomeninges. Lotti (20) reports a case of recurring tuberculous meningitis in a 22 year old patient who had attacks of greater or less severity since the age of 10 years. The occasional dissemination of tubercle bacilli and their products from a caseating tuberculoma of the brain into an allergic space would result in an acute exudative meningitis. Our experiments, taken with the fact that most if not all cases of tuberculous meningitis are secondary to some primary focus in another part of the body, assuredly indicate that a state of allergy exists in the subarachnoid spaces before the bacillus reaches the boundaries of the brain or its coverings.

Hypersensitive states of tissues are known to exist in infectious diseases other than tuberculosis. Studies pertaining to these phenomena in other diseases and their relation to certain central nervous system complications, particularly the complications following influenza, pneumonia, vaccination, etc., are in process of investigation.

SUMMARY

1. When living or dead tubercle bacilli and their products are placed in direct contact with the leptomeninges of hypersensitive (tuberculous) animals, there is a definite clinical and pathological response.

2. The clinical response is characterized by an onset of weakness, twitchings, convulsions and death of the animal within 6 to 12 hours.

3. Histologically the central nervous system shows an extensive

polymorphonuclear exudate distributed throughout the subarachnoid spaces of the brain and extending into the perivascular spaces.

4. The intensity of the response is directly proportional to the quantity of visceral tuberculosis or to the dose of tuberculin employed.

5. When small quantities of tuberculin are employed so as to permit the animal to survive longer than 24 hours, there is an exudate found in the sulci and at the base of the brain which is characterized by small lymphocytes.

6. The non-tuberculous animals when inoculated with tuberculin or tubercle bacilli revealed no clinical or pathological response. The tuberculous animals, on the other hand, when inoculated with glycerine broth always responded by a definite but slight polymorphonuclear exudate.

7. The possible relationship of the allergic state to postinfectious complications of the central nervous system is discussed.

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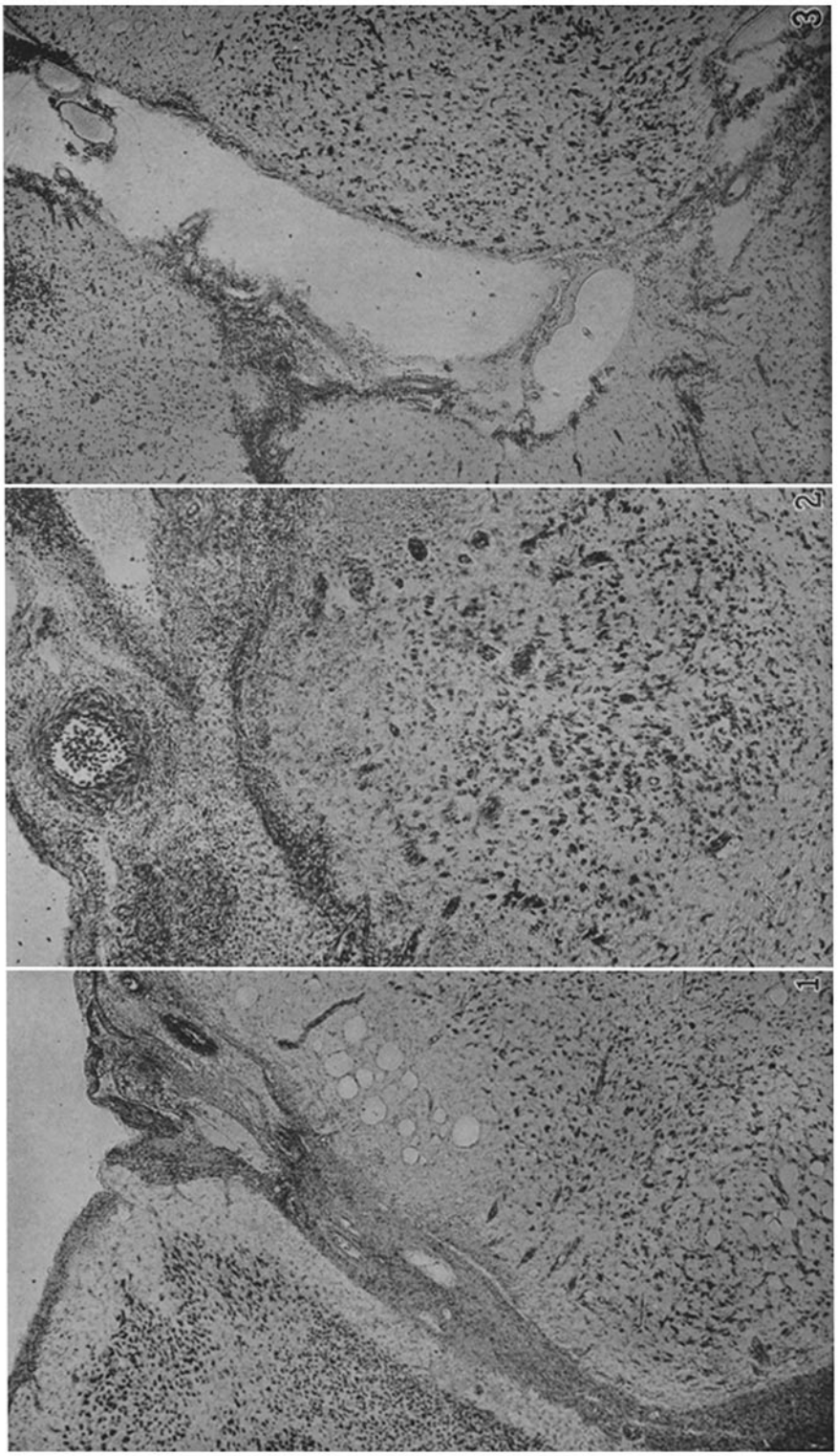
EXPLANATION OF PLATE 10

FIG. 1. Section of brain from a tuberculous guinea pig that received 0.3 cc. of 1/100 dilution of tuberculin. The Sylvian fissure is distended and densely compact with a cellular exudate. × 45.

FIG. 2. Exudate at base of brain with perivascular and subpial infiltration. × 70.

FIG. 3. Section of brain from a tuberculous guinea pig that received glycerine broth *via* basal cistern. Slight cellular response along borders of Sylvian fissure. × 45.



(Burn and Finley: Hypersensitivity and meningitis. I)