provided the usual precautions in respect to allergic reactions to the drug were observed.

As may be noted from Table I, a significant blood penicillin level was present in 24 out of 26 cases with chronic suppurative sinusitis following the intra-antral instillation of 200,000 units of the crystalline product. In every instance except one (Patient No. 25) the blood level was uniformly higher after the addition of hyaluronidase to the penicillin solution. In general, the blood levels were found to be about 2 to 3 times greater than those found without its use. Furthermore, it is of interest that patients No. 19 and No. 21 in whom no evidence of absorption could be demonstrated, showed significant levels after the addition of hyaluronidase. These findings suggest that hyaluronidase increases diffusion and penetration of penicillin through the diseased antral mucosa.

Similar findings to those noted above were obtained in subjects with non-diseased mucous membranes (Table II). A significant level was found in the blood of all 5 subjects after the antral instillation of penicillin and in each patient tested, the addition of hyaluronidase resulted in higher levels.

Although we were not primarily concerned in this study with the evaluation of the clinical effectiveness of large doses of crystalline penicillin supplemented by hyaluronidase in the treatment of chronic suppurative disease of the maxillary sinus, it is of interest that marked clinical improvement occurred in some of the patients.

Summary. The instillation of 200,000 units of crystalline penicillin G into the diseased and normal paranasal antrum is well tolerated and except for the development of an allergic reaction in one patient, was without any adverse effect. In 24 out of 26 patients with chronic suppurative disease of the sinuses and in all 5 normal subjects, a significant penicillin level in the blood was found after the intra-antral instillation.

In both groups, the addition of hyaluronidase to the instilled penicillin resulted in even higher blood levels than those found without its use, with one exception. In two patients in whom no blood level could be demonstrated, the addition of hyaluronidase resulted in a significant concentration of penicillin in the blood. It is postulated that the increased blood penicillin level following hyaluronidase is due to greater diffusion and penetration of the penicillin as a result of the spreading action of hyaluronidase.

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Tuberculin Reaction. III. Transfer of Systemic Tuberculin Sensitivity with Cells of Tuberculous Guinea Pigs.*

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In spite of many years of research by numerous investigators the passive transfer of tuberculin sensitivity was not accomplished with any degree of certainty or regularity until the important work of Chase[1] whose technic has provided a new means of exploring many aspects of this highly important type of sensitivity.†

In previous publications,[2,3] we have pre-

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1 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

† The term sensitivity is used throughout the present article in preference to the more commonly used term hypersensitivity.

sented experimental evidence which indicates that the tuberculin reaction is the result of the interaction of a sessile antibody with specific antigen. In this work[2] the observation of Chase[1] that cutaneous tuberculin sensitivity can be passively transferred with the cells of induced peritoneal exudates of non-tuberculous donor guinea pigs sensitized with heat-killed tubercle bacilli was confirmed by the use of tuberculous donors. Another observation was that the *in vitro* effects of tuberculin in suppressing the initial migration of leukocytes from splenic explants of tuberculous guinea pigs is markedly reduced by specific desensitization.[3] In addition the results of preliminary trials were presented which indicated that homologous passive transfer of systemic tuberculin sensitivity can be accomplished in the guinea pig with cells of peritoneal exudates of tuberculous donors.[2]

The results given in the present report confirm and extend the observations of these preliminary trials.

Although the systemic tuberculin reaction was recognized as early as the local cutaneous reaction,[4,5] and is still used in the standardization of Old Tuberculin, it is not employed as widely as the cutaneous reaction for determining tuberculin sensitivity in experimental tuberculosis of laboratory animals. It is unfortunate that the systemic reaction commonly involves the sacrifice of these animals, since it is probably a more reliable measure of tuberculin sensitivity than the cutaneous reaction which is observed to be non-specifically affected by many factors such as age,[6,7] general health and intercurrent disease.[8-11]

Koch[4,5] observed that fatal systemic tuberculin shock in the guinea pig is so characteristic that there is little reason to confuse it with other types of shock. The principal features which Koch noted to characterize the reaction were the delayed onset, the protracted course with extreme malaise and the focal reactions at the site of tuberculous lesions.

In his studies on systemic tuberculin shock in the guinea pig Weinzirl[12] was also much impressed by the inactivity of the animals, the limpness of their muscles, and the anorexia and general prostration they exhibited. He proposed that the experimental provocation of the systemic tuberculin reaction by the intraperitoneal injection of tuberculin be called the "systemic test".

The systemic tuberculin reaction has been used in the past as an index of sensitivity in many of the numerous attempts that have been made to accomplish homologous and heterologous passive transfer of tuberculin sensitivity in laboratory animals and man.[13-23] In these attempts a variety of transfer materials were used such as serum, whole blood, exudates, transudates, and organ mashes. The

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3 Kirchheimer, W. F., and Weiser, R. S., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 407.

4 Koch, R., *Dtsch. Med. Wchnschr.*, 1890, **16**, 1029.

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12 Weinzirl, J., *Tubercle*, 1931, **12**, 488.

13 Friedemann, V., *Münch. Med. Wchnschr.*, 1907, **49**, 2414.

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17 Joseph, K., *Beitr. z. Klin. der Tuberk. von Bauer*, 1910, *Bd.* **17**, 461.

18 Yamanouchi, T., *Zentralbl. f. Bakt.*, 1909, Part I, **44**, 434.

19 Yamanouchi, T., *Comp. Rend. Soc. Biol.*, 1909, **66**, 531.

20 Onaka, M., *Beitr. z. Klin. der Tuberk. von Bauer*, 1910, Orig. VII, 507.

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22 Massol, L., Breton, M., and Bruyant, L., *Comp. Rend. Soc. Biol.*, 1913, **74**, 185.

23 Selter, H., *Z. f. Immunitatsforschg.*, 1921, **32**, 325.

results of these various experiments were irregular and inconclusive.

Methods. In the present investigation the guinea pigs employed as cell donors weighed between 650 and 800 g and were sensitized 5-9 weeks prior to use by the intraperitoneal injection of approximately 5 mg of living *Mycobacterium tuberculosis* var. *bovis* of the B.C.G. strain. Only those animals showing necrotic reactions to the intradermal injection of 0.1 ml of 1:100 O.T. were used as donors. Cell exudates were induced in the donor animals as outlined by Chase[1] by the intraperitoneal injection of 25 to 30 ml of paraffin oil.‡ The animals were killed 48 hours later and the cells collected and washed by centrifugation in heparinized guinea pig serum—Tyrode solution in the manner described by Chase.[1]

The recipients were large, healthy male guinea pigs each of which was injected intraperitoneally with the washed, pooled cells from 6 donors. Each recipient was challenged 48 hours after receiving the donor cells by the intraperitoneal injection of 2 ml of O.T. (1X International Standard) diluted with 8 ml of 0.85% NaCl.§

The controls consisted of recipients given intraperitoneal injections of comparable quantities of cells from (1) normal donors, (2) donors sensitized 5 to 9 weeks previously with 5 mg of living *Mycobacterium smegmatis*, (these animals gave 3+ cutaneous reactions to smegmatis tuberculin 1:100) (3) donors injected 5 to 9 weeks previously with powdered quartz and (4) tuberculous donors desensitized by the repeated subcutaneous injection of increasing doses of O.T. in the manner previously described.[3] These controls were challenged with O.T. in the manner described above. An additional control group consisted of recipients given the cells of sensitive donors and challenged with a dose of diluted glycerine broth comparable to the concentration of these substances present in the O.T. preparation used.

The peritoneal cells of the sensitive donors and the control groups, including desensitized animals, were examined by the neutral red supravital method as outlined previously.[2] A large percentage of the cells of all of the preparations were found to be living.

Except for the development of slight abdominal tenderness in an occasional animal, all recipients appeared to remain physically normal during the 48 hour period prior to the challenging dose of shock test substance. Their rectal temperatures remained at normal levels.

Results. The results of the principal experiments are recorded in Table I. They show that of the 8 recipients of cells from sensitive animals, 5 died of tuberculin shock in from 20 to 50 hours, and 3 were severely shocked but recovered. Among the control groups one animal developed tuberculin shock.

A mild, but transient discomfort was observed in most of the animals immediately following the challenging injection of tuberculin. This was apparently due to the glycerine, since the same effect was observed following injection of normal animals with similar dilutions of glycerine and likewise in the group of recipients of the cells of sensitive animals challenged with diluted glycerine broth.

One of the recipients of cells of desensitized tuberculous donor animals developed mild symptoms of tuberculin shock and recovered. Another animal of this group showed no symptoms of shock following the challenging dose of O.T. but suddenly died at the 52nd hour. There was no indication at autopsy as to what the cause of death may have been. It seems unlikely that it was due to tuberculin shock since the characteristic symptoms of tuberculin shock were lacking.

All of the recipients of the cells of sensitive donors developed typical systemic tuberculin shock which usually began after an interval of about 3 hours and became severe by 4 to 5 hours. The first evidence of shock was the development of muscle weakness ruffled hair and increased respiration. In all instances a marked drop in body temperature

‡ The mineral oil used was Bayol F. distributed by Stanco Incorporated, 2 Park Avenue, New York City.

§ The Old Tuberculin used in these experiments was kindly supplied by the Lederle Laboratories of Pearl River, N. Y.

TABLE I. Passive Transfer of Systemic Tuberculin Sensitivity with Cells of Induced Peritoneal Exudates of Tuberculous Guinea Pigs.

Recipient No.	Preparation of donors	Sensitizing dose of cells in ml	Dose of challenging material	Reaction of animal
1	B.C.G. sensitized	.5	10.0 ml diluted O.T.	Died at 30 hrs.
2	"	.3	" " "	" " 21 "
3	"	1.0	" " "	" " 21 "
4	"	.5	" " "	In severe shock at 4 hrs, fully recovered at 30 hrs.
5	"	.5	" " "	Died at 20 hrs.
6	"	.5	" " "	Severe shock at 4 hrs, fully recovered after 48 hrs.
7	"	.8	" " "	Severe shock at 5 hrs, fully recovered after 48 hrs.
8	"	.5	" " "	Died at 50 hrs.
Controls				
1	*M. smegmatis* sensitized	.5	" " "	No symptoms.
2	B.C.G. sensitized and desensitized	1.8	" " "	Slight symptoms shock 4 hr, fully recovered 10 hr
3	" "	.6	" " "	No symptoms.
4	" "	.7	" " "	No symptoms, but died suddenly after 52 hrs.
5	Quartz treated	.5	" " "	No symptoms
6	"	.8	" " "	" "
7	Normal	.5	" " "	" "
8	"	1.2	" " "	" "
9	B.C.G. sensitized	.9	10.0 ml diluted glycerine broth	" "
10	"	.5	" " "	" "

occurred, which in fatal cases went as low as 34°C just prior to death. In fatal cases the severity of shock increased progressively until death and marked abdominal tenderness was observed to develop in many of the animals during the late stages of shock. In those that survived, recovery from the early symptoms of shock usually began about the 20th hour and continued gradually with the result that shock symptoms disappeared entirely by about the 48th hour.

All of the animals that died were promptly autopsied. A small amount of clear yellowish and at times sanguinous fluid was commonly found in the peritoneal cavity. The omentum was rolled up and the omental folds adherent. A small amount of fibrinous deposit was occasionally present on the surface of the liver. The mesenteric vessels appeared to be engorged and a pronounced reddish discoloration of the walls of the small intestines was found with great regularity. The liver was large and distended with blood. Bacteriological examinations of the peritoneal fluid by smear and culture were negative in all instances. There were no other abnormal findings.

Summary. The results of the present investigations establish that systemic tuberculin sensitivity can be passively transferred in the guinea pig with the cells of induced peritoneal exudates of tuberculous donors. The systemic shock produced in recipients passively sensitized in this manner is of the typical delayed tuberculin type. These findings supplement the observation of Chase[1] that cutaneous tuberculin sensitivity can be passively transferred in the guinea pig with the cells of induced peritoneal exudates of donors sensitized with heat-killed tubercle bacilli by providing additional evidence that the sensitivity transferred is of the tuberculin type. In a limited number of trials it was also found that desensitization of tuberculous donors with tuberculin tends to abolish the capacity of their peritoneal cells to passively transfer tuberculin sensitivity. If this observation is confirmed it will provide strong evidence that desensitization results from the saturation of fixed cellular antibody with antigen.