A PYOGENIC FILTERABLE AGENT IN THE
ALBINO RAT

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PLATE 17

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This paper describes, more fully than our preliminary communica-
tion (1), a filterable agent resembling the viruses which produces
extensive necrosis and suppuration in certain tissues of the white rat.

One of us (2) has been carrying on for some years a search for antibodies
against sarcoma 39 in rats which had cured themselves of this neoplasm, mixing
emulsions of the tumor before inoculation with various organs of the rat or with
adsorbents employed to remove an antibody from tissue extracts, or incubating
them in such extracts or in Locke-Ringer solution. Though all materials had
been proved sterile before use by ordinary bacteriological methods, large abscesses
sometimes developed within a few days at the site where these tumor emulsions
had been injected. This complication, which arose about twenty times in some
100 instances at irregular intervals throughout the course of five or six years, was
at first ascribed to bacterial contamination, for which the requisite manipulations
gave abundant opportunity. Aerobic and anaerobic cultures of the pus, however,
were repeatedly negative in plain broth, dextrose broth, streptococcus broth,
Rosenow's brain broth, Loeffler's blood serum, Huntoon's hormone medium,
plain agar, blood agar, dextrose agar slants, Petroff's egg medium, blood plates
in 10 per cent carbon dioxide, and the Bordet-Gengou medium. Organisms
could not be found in smears stained after the methods of Gram, Wright, Ziehl-
Neelsen, or with polychrome methylene blue, and, finally, no spirochetes were
discovered upon dark field examination.

This consistent failure could not but suggest the participation of something
resembling a virus, yet a search of the literature revealed only one virus indig-
enuous to the rat—reported by Novy (3) and quite different from the one now
under discussion; and none in any animal species with activities so eminently
pyogenic.

The Lesions in the White Rat

We have employed as standard material a 10 per cent tissue extract, made by
grinding pus and abscess wall with sand in the appropriate amount of Locke-
Ringer solution without glucose, and centrifuging at moderate speed for 5 minutes. The customary dose has been 0.05 cc. of the supernatant fluid.

Subcutaneous Injection.—About 24 hours after inoculation there appears a slight puffiness which can be seen but not yet felt, and if the hair be thin some slight reddening may be discerned. On the 2nd day there is a palpable swelling, and by the 3rd its inflammatory nature is clearly apparent. The skin is hyperemic, the underlying tissue thickened, the outline diffuse, and the lesion about 2 cm. in diameter. At this time, though generally a few days later, the abscess may open and discharge its contents on the surface, an event which bears no relation to its size for smaller ones may evacuate spontaneously in this manner while larger ones may never do so. Many of the lesions, in fact, are absorbed under an unbroken skin during the course of several weeks.

In rats autopsied 24 hours after inoculation the subcutaneous tissues about the site are found to be slightly thickened and intensely congested, while through them runs a long whitish streak as though they had been seared by a hot wire, representing, no doubt, necrosis along the needle tract. On the 2nd day conditions are about the same except that this streak is now broader. On the 3rd the connective tissue is much more definitely hyperplastic, having somewhat the appearance and consistency of a rather soft fibroma, but in most cases free pus has not yet appeared. At this stage of the process the regional lymph nodes may begin to enlarge.

By the end of the first 24 hours the congestion has reached its height, for it is no more intense on the 2nd or 3rd day than on the first. On the 4th day (Fig. 1) free pus may or may not be seen but by the 5th or 6th it is uniformly present. An abscess removed at this time with its wall will weigh from 0.7 to 1.5 gm., about one-third of which will be accounted for by thick greenish yellow pus. By the 10th day the proliferative process is regressing and the abscess has become a thin walled pus sac (Fig. 2).

The amount of extract administered is immaterial within reasonable limits, doses of from 0.025 to 0.80 cc. having all produced large characteristic lesions. Even the highest of these doses caused no evident constitutional symptoms.

After the usual 0.05 cc. the animals move about normally, eat well, retain a glossy coat, gain weight, and none die. There does take place, however, a definite increase in the proportion of circulating polymorphonuclear leucocytes on the day after injection from the 20 per cent or so found in health to some 60 per cent, with a gradual return to normal during the next 7 days. The lymphocyte count, meanwhile, tends in the opposite direction. However, because of the widely recognized difficulty in obtaining reliable blood counts for the rat and the mouse, it is safer not to attach too much significance to these results for the present.

To preclude any chance that we were dealing with some sort of irritative lesion elicited merely by the products of tissue degeneration,
sterile abscesses were produced in rats by the subcutaneous administration of turpentine, removed after intervals of 7 or 10 days, and inoculated in the form either of tissue fragments or of 10 per cent extracts. Among 9 such lesions, injected into 18 rats, not a single one induced a response comparable in any way to that set up by the agent. Similarly, the introduction of agent which had been inactivated by heat produced no macroscopic changes.

Intravenous Injection.—Introduction of this filterable agent into the blood stream in the form of tissue extract may be attended by consequences far more serious than those following its subcutaneous administration.

Seven groups of rats containing 42 animals in all were injected by way of a caudal vein with 0.5 cc. of extract. They remained in apparent good health for the next 2 or 3 days, but on the 4th redness and swelling of one or more paws began to appear in most of the group, a characteristic lesion which is illustrated in Fig. 3. By the 7th day, or thereabout, some 20 per cent of the animals were moving themselves around by the forelegs, with paralyzed hindlegs trailing behind in the supine position, a condition of which the cause has not yet been determined.

Within a day or so after injection there often set in an emaciation which progressed so rapidly that about one-third of the body weight was lost in 1 or 2 weeks, though how much of the decrease was due to the infection itself and how much to fasting entailed by difficulty in getting at the food supply cannot be decided at present. That the latter may play an important rôle is suggested by the empty gastrointestinal tract often encountered at autopsy, a condition which cannot be referred to distaste for food because the animals ate eagerly when fed from the hand.

In addition to these abscesses in the feet, collections of pus were found in the testis, about the head, or in the soft parts surrounding the large joints, a more or less characteristic site being the subcutaneous tissues cephalad to the scapula. This localization, curious because no lymph node occupies this locality in the rat and no connection with the hibernating gland could be demonstrated, occurred in 5 animals of the intravenous group and in 2 out of 20 inoculated in the testis, or in 7 among a total of 62, an incidence too high to be explained away by pure chance. Furthermore, 3 rats had such an abscess at identical points on the two sides.

In a few instances intravenous inoculation caused no apparent disturbance of health; or, again, a rat that seemed hopelessly ill would eventually recover. The mortality for the group as a whole was about 25 per cent.

The rectal temperatures in one lot of 12 animals, taken 3 days after inoculation, were found to range from 98.8° to 101.8°, being above 100° in 9 instances, while 3 days later none had a temperature higher than 100.6° despite the presence
of abscesses in the paws or about the large joints. In 12 normal rats investigated at the same time a range of 97.6–98.8° was found.

*Intratesticular Inoculation.*—The testis is a vulnerable site, injection of a 1:1,000 dilution of the extract resulting in its total destruction by suppuration.

The introduction of 0.2 to 0.4 cc. of 10 per cent extract into a testis in 21 rats induced redness and swelling with eventual suppuration, the process becoming apparent about 2 days after inoculation and terminating in recovery by evacuation or absorption of the abscess during the following few weeks. As in the case of intravenous inoculation, abscesses developed in the feet, in the soft tissues about a large joint, or at various points in the subcutaneous tissues, including the region cephalad to the scapula.

*Intraperitoneal Inoculation.*—The introduction of filterable agent by this route causes no apparent constitutional disturbance.

Of 12 rats thus injected with 0.5 cc. of extract none died. Those killed for examination from 4 to 15 days later had abscesses along the needle tract, and in 3 instances the testis on the inoculated side was involved, probably by extension of the suppurative process. Occasionally an abscess was discovered in the gastrosplenic or the great omentum, but never was there any generalized peritonitis. In 1 animal a small collection of pus was found in the subcutaneous tissues at the root of an ear, showing that the agent can affect a distant site after intraperitoneal injection, though it does not appear to do so as a rule.

*Intracerebral Inoculation.*—The results of intracerebral injection resemble somewhat those following inoculation by way of the blood stream.

Of 12 rats that had filterable agent introduced into the brain 3 developed the characteristic red and swollen feet and 1 of these the paralysis. By the 4th day all had abscesses at the inoculation site in the subcutaneous tissues overlying the frontal bone, and in 3 that were killed for autopsy 18 and 22 days after inoculation an intracranial abscess was discovered which had evidently arisen by extension of the superficial lesion along the needle tract. One of these had been sacrificed because it was found biting at a swollen hind foot, its behavior recalling in certain measure the reaction of animals to the intense itching of infectious bulbar paralysis, as described by Hurst (4).

Four of the rats suffered no apparent effect from the inoculation save for the subcutaneous abscess in the frontal region, 4 died and were not autopsied, and 1 with inflamed feet recovered.
Percutaneous Inoculation.—The epidermis is exempt, a fact which may explain our failure to observe spontaneous transfer of the disease from infected to healthy rats.

Two drops of extract were rubbed thoroughly with a glass rod into the shaved and scarified skin of 6 rats. The abrasions were healing, or entirely healed, 4 days later with no sign of suppuration.

Distribution of the Filterable Agent

As the heart's blood elicited the typical abscess when withdrawn 7, 11, or 14 days after intravenous or intratesticular administration and subcutaneously injected into other rats, a wide distribution of the agent was only to be expected under these circumstances. Inoculation of various organs from rats infected by either of these two routes confirmed this anticipation for, with the exception of the suprarenal gland, the filterable agent was discovered at one time or another in all those tested; namely, the spleen, lumbar nodes, kidney, liver, and brain. It was not found, however, in the urine. On the other hand, 32 days after intravenous injection it had disappeared from the blood, lumbar nodes, liver, and kidney, only spleen and brain now producing suppurative lesions.

A more precise way of determining its tropism was to test the organs from rats with subcutaneous abscesses.

From the 1st to the 5th week following subcutaneous injection various organs were removed from 45 rats, belonging to different passages, and introduced under the skin in 685 others to see which would elicit the characteristic abscess. Where size permitted, as in the case of the spleen, the organ was reduced to a paste and injected in amounts of 0.10 cc., while smaller structures such as the suprarenal glands were halved and inoculated with a hollow needle. As experience had shown that sodium citrate did not interfere with the activities of the agent the heart's blood was drawn into a syringe containing a small crystal of this salt; here the dose ran from 0.4 to 1.5 cc. according to the amount available. The number of animals inoculated with each sample was dictated by its quantity. Emulsions of the larger organs sufficed for 6 rats, grafts of the lymph nodes for from 2 to 4, and of the suprarenal glands for 4.

The right axillary nodes were selected for testing because they were on the same side as the lesion yet not, like the lumbar group, imbedded in it, and the left axillary and the lumbar nodes because they represented distant ones.
As Text-fig. 1 shows, the agent has a distinct predilection for the lymph nodes and the brain, not having been found elsewhere save occasionally in the spleen and suprarenal gland. It is particularly worthy of note that although relatively enormous quantities must have been present at the site of the abscess, and some must have been distributed to the rest of the body, as attested by its recovery from distant organs, not once could it be demonstrated in sarcoma 39, though 17 neoplasms were examined from 4 to 34 days after their bearers had received a subcutaneous injection of filterable agent on the opposite side. The idea promptly suggested itself that this failure might indicate neutralization of the filterable agent within the sarcoma rather than failure to localize in the neoplasm, but it was abandoned for the time being when a tumor emulsion purposely incubated with agent was found to have caused no inactivation.

It should be pointed out that filterable agent was absent from, more often than present in, the organs tested; thus, for example, it was discovered in only 2 out of 23 spleens. These negative results constituted an automatic control, and obviated the necessity of examining organs from normal rats in order to preclude the possibility of a generalized infection among the animals of our strain.

Pathology

Tissues destined for microscopic examination were fixed in Zenker's fluid and stained with hematoxylin-eosin or with eosin-methylene blue. Subcutaneously injected, the filterable agent causes widespread necrosis within 24 hours, soon followed by a monocytic reaction, permeation by polymorphonuclear leucocytes, and, after 4 or 5 days, the formation of a large densely encapsulated abscess. In the corium these changes are accelerated, and discrete pus may be found as early as the 1st or 2nd day (Fig. 4). We refer to the filterable agent as pyogenic because this suppurative lesion is its salient effect in the gross.

As for the internal organs, the only constant microscopic alteration was a lively reticulum cell reaction affecting the regional lymph nodes in the case of subcutaneous inoculation, or more generally distributed after intravenous administration. Throughout the remainder of the body, even when filterable agent had been introduced into the circulation, no histological changes were discovered which could not be
Text-FIG. 1. Distribution of the filterable agent after subcutaneous inoculation.

■, agent present. □, agent not found. □, material not examined.
duplicated in presumably normal rats taken from stock. In particular, no perivascular infiltration was seen in the brain, no matter what the site of inoculation.

Nerves in the vicinity of subcutaneous abscesses showed no visible damage in sections stained with hematoxylin and eosin.

The supplicative lesions in the feet involved the soft parts only, the bone itself being spared though the marrow was thickly strewn with polymorphonuclear leucocytes.

*Inclusion Bodies.*—In the epithelium overlying abscesses of from 12 to 24 hours' duration a small number of eosinophile cytoplasmic structures have been found (Figs. 5 and 6) which closely resemble Guarnieri bodies, but Giemsa's and Goodpasture's stains, hematoxylin-eosin, and eosin, rosanilin, or phloxin in combination with methylene blue all failed to disclose inclusion bodies in sections of the various internal organs, of subcutaneous or intradermal abscesses, or of chick chorio-allantoic membrane or rabbit cornea inoculated with the agent. Though search has been made at intervals of from 2 hours to many days after inoculation it may be that the most propitious time has yet to be found.

*Susceptibility of Other Species*

**Mouse.**—This animal is particularly susceptible.

Twelve mice were inoculated subcutaneously, each with 0.05 cc. of extract, with the result that from 2 to 4 days later 9 had massive edematous swellings involving the whole side. Unlike rats injected in the same way these animals looked acutely ill, and most of them died within a week. In 2 that were killed for autopsy on the 3rd day the subcutaneous tissues were found hemorrhagic and necrotic, though without free pus, while microscopic examination revealed extensive permeation by polymorphonuclear leucocytes and a widespread necrosis. One animal lived until the 15th day, when it was sacrificed because it was moribund with an extensive abscess at the inoculation site.

Intracerebral injection in the mouse is followed almost invariably, on the 3rd day at the latest, by extensive edema of the head with suppuration in the subcutaneous tissues overlying the frontal region, where the needle entered the skull, and death generally ensues after from 4 to 11 days. Microscopic examination of the brain has revealed neither suppuration nor perivascular infiltration, the visible damage having been limited to a slight meningitis.

The filterable agent has been transmitted for 6 passages in the mouse brain, apparently with some loss of virulence for the rat at the end of the series.
Mice infected by nasal insufflation usually died of septic pneumonia after from 5 to 17 days, only 3 out of 12 having escaped. The brains from 2 of these survivors, inoculated subcutaneously into rats and mice, elicited no abscesses; hence, in all probability, filterable agent had not reached the brain as a consequence of the insufflation.

**Guinea Pig.**—Unlike the mouse, this species is highly refractory.

Following the subcutaneous introduction of 0.4 cc. of extract minute nodules, a few millimeters in diameter, appeared in 2 of the injected animals, while the other 2 developed no macroscopic reaction at the inoculation site.

**Rabbit.**—Subcutaneous injection is somewhat more successful than in the guinea pig.

Thus the administration of 0.5 cc. of extract produced slightly red flat tumefactions in all 4 rabbits inoculated by this route. These lesions were beginning to regress by the 4th day and were entirely healed after about 2 weeks. In 1 animal, killed for examination on the 6th day, the subcutaneous tissues appeared to be the seat of suppuration, though no free pus was found. The microscope revealed extensive leucocytic permeation.

A similar dose, introduced into the blood stream in 2 rabbits, caused no appreciable disturbance, while intracerebral inoculation with 0.4 cc. appeared equally harmless in the 1 rabbit thus injected.

Dropped upon the scarified cornea in 2 rabbits, the extract produced only conjunctivitis, which began to clear up within a week.

**Chick Embryo.**—A mouse strain has been maintained on the chorioallantoic membrane of the hen’s egg, now for 10 generations, and is uniformly fatal for the mouse upon intracerebral inoculation. The presence of demonstrable agent may or may not be associated with the development of pin-point non-suppurative opacities on the membranes.

**Immunity**

Immunity, which is fairly well established by the 7th day after inoculation of the filterable agent, lasts for about 3 months, though it begins to diminish after 2.

Fifty-five rats that had recovered from intradermal, subcutaneous, intraperitoneal, or intratesticular inoculations were given a second injection from 7 to 101 days after the first one, the testing dose being administered subcutaneously and, in the case of those animals that had already received a subcutaneous or an
intradermal inoculation, on the opposite side of the body. About half the group proved to be entirely refractory and the remainder more or less so, for the resulting abscesses had a diameter exceeding 0.5 cm. in only 8; in 4 of these they attained the size of lesions in previously uninoculated rats.

This immunity was not accompanied by demonstrable neutralizing factors in the blood in 3 instances where signs of its presence were sought.

Serum from rats with 13 day old abscesses was combined with equal parts of a 1:100, 1:1,000, or 1:10,000 dilution of a 10 per cent saline extract, a similar series being set up with normal serum. After 90 minutes’ incubation at 37°C. these mixtures were inoculated intratesticularly, together with appropriate controls of serum or undiluted extract alone. When the animals were examined 5 days later there was no definite evidence of neutralization.

A second test, with 11 day and 24 day citrated plasma, and a third with 8 day serum, were just as unsatisfactory, though in one of these the mixtures were kept in the ice box overnight following their incubation, and the testing inoculation was made at a different site, namely, in the subcutaneous tissues.

A macroscopic test for precipitins in 17 day and 24 day citrated plasma was negative.

An 0.5 per cent phenolized vaccine did not confer immunity in 5 rats treated with it.

Properties of the Filterable Agent

Filterability.—The agent can be passed through a Berkefeld N filter, though with considerable difficulty. In the preliminary account we said that it would traverse a W candle, but we have been unable to confirm this first result. The filter, used in connection with a water-tap aspirator of the common type, was impervious to the meningococcus both before and after the agent had passed through it, and the filtrate produced abscesses in 4 out of the 6 rats inoculated with it, but 4 subsequent attempts with a new W candle were entirely unsuccessful. Indeed, the agent will not always pass a Berkefeld N.

The attempts to filter through an N candle were made with lesions of different ages, extracted either in Locke-Ringer or in physiological saline solution. As a test of the candle, cultures of Staphylococcus pyogenes albus in plain broth were employed. In some experiments the culture was added at the beginning of the filtration; in others, when filtration had been half completed in order that the effect of the acid culture might be observed; while in still others plain broth was
added when filtration was started, or when it was half over, the test culture being employed at the end of the process. None of these variations made any difference in the outcome.

In 2 experiments the agent did not pass the N candle at all, for the filtrate elicited no lesions in 18 rats and 3 mice subcutaneously inoculated or in 3 mice intracerebrally injected. In 3 other trials the filtrate produced small abscesses in 9 out of 30 rats to which it was administered subcutaneously and in all 3 that received an intratesticular inoculation, or in 12 out of 33 altogether.

**Infectivity.**—Titration showed that a 10 per cent extract could be diluted to $10^{-2}$ without loss of infectivity. At $10^{-3}$, $10^{-4}$, and $10^{-6}$ some individual resistance began to appear in the injected rats, for in each of the 3 groups 1 animal was negative while the remaining 6, 2 in each lot, had small though characteristic lesions. At $10^{-6}$ and $10^{-7}$ no abscesses were produced.

**Viability.**—The filterable agent is attenuated by 30 or 60 minutes' exposure to 56°C., for extracts so treated did not elicit quite so many or quite such large abscesses. It is killed by heating for 1 hour at 60°C.

To ultraviolet light it is moderately susceptible. With a Cooper-Hewitt 110 volt, 4 ampere, mercury vapor lamp distant 20 cm. an exposure of 1 minute appeared to weaken it a little, while after 15 and 30 minutes all virulence was lost.

In two experiments the agent was killed by 0.05 per cent formol overnight at room temperature (22°C.).

Under the same conditions, 0.05 per cent phenol had no demonstrable effect, but 0.5 per cent abolished all activity.

Desiccation *in vacuo* after freezing weakened the agent but little, tissues so treated producing typical abscesses immediately afterward and at the end of a 45 day sojourn in the ice box. 5 rapid freezings at about $-70^\circ$C., interspersed with thawings at $37^\circ$C., had no deleterious effect.

No loss of infectivity was observed in a saline extract stored in the ice box for 10 or 21 days, but at the end of 6 weeks the agent had become so attenuated as to cause rather small abscesses in only 2 out of 6 rats inoculated, and when tested after 78 and 83 days it was found entirely inert. Full virulence was retained by a rat abscess kept in 50 per cent glycerol-saline for 31 days under the same
conditions of temperature, but there was some loss after 47 days and total abolition after 73 days. Infected mouse brain was virulent after cold storage in glycerol-saline for 32 days, but not after 45 and 60 days.

The agent is not susceptible to oxidation, under the conditions employed, the passage of filtered washed air through an extract at moderate speed for an hour having left its virulence unimpaired.

DISCUSSION

Relation of Filterable Agent to Tumor

During some 20 years' routine transplantation of rat sarcoma 39 no evidence of pus has ever been observed, purulent lesions having appeared only when the neoplasm had been treated in some way before inoculation, and then only from time to time. Thus abscesses followed the injection of tumor emulsions that had been incubated in tissue extracts prepared with Locke-Ringer or physiological saline solution, or with distilled water; of an emulsion that had been incubated in Locke-Ringer solution alone; and of one that had merely been combined with charcoal employed to adsorb antibody from tissue extracts. Apparently incubation, the various salts in the solutions, and the presence of a tissue extract were none of them a prerequisite, and the mystery was deepened by the fact that suppuration might appear in one of two experiments done under what were thought to be identical conditions and not in the other. A few preliminary efforts to elicit pus with sarcoma 39 under controlled conditions have failed. Emulsions of this growth incubated in various media, or oxidized by the passage of air, have produced no abscesses so far, but as the circumstances under which the pyogenic agent appears and reappears constitute one of its most interesting features a continuation of these endeavors may be worth while.

In any case there is no evidence so far to suggest a specific affinity between this agent and sarcoma 39, as has already been explained in discussing the text-figure. It is true that from 4 to 15 days after the agent had been injected into the blood stream or the testis in tumor-bearing rats it could be recovered uniformly from this sarcoma and from 4 other propagable new growths—sarcoma 8, the Walker carcinosarcoma 256, the Flexner-Jobling carcinoma, and a benzpyrene
sarcoma—but following either type of administration it was sometimes demonstrable also in the blood, the liver, or the kidney. As neither these organs nor sarcoma 39 were found to contain the agent after subcutaneous inoculation, its transfer to them from the bloodstream was probably an outcome of mere propinquity rather than of any definite attraction. Its uniform presence in tumors after intravenous injection, as contrasted with its occasional presence in the organs, is more easily explained by the favorable circumstances they provide for viruses in general than by any specific affinity between this agent and neoplastic tissue. Thus, for example, Levaditi, Schoen, and Reinié (5) have shown that rabies street virus will live in the cells of the Brown-Pearce rabbit carcinoma, Syverton and Berry (6) that the Shope papilloma can be infected with several viruses, and Rivers and Pearce (7) that the Brown-Pearce carcinoma will carry virus III and vaccinia virus, both of which survive there for a longer period than in normal tissues.

It may be mentioned in passing that the pyogenic agent could not be recovered from a Shope papilloma after injection into an ear vein of a rabbit bearing this growth.

When the agent was discovered in a tumor or an organ subsequent to intravenous or intratesticular inoculation it was not by virtue of any blood retained therein, for instances occurred where the agent was found in the liver, kidney, or neoplasm from an exsanguinated animal but not in the heart’s blood itself.

The possibility of a cross-immunity between this filterable agent and sarcoma 39 has been investigated with some care. Rats that had recovered from an infection with the agent were discovered to be fully susceptible to the sarcoma, while rats that bore the tumor or had cured themselves of it and been proved refractory on second grafting showed no resistance to the filterable agent. Furthermore, introduction of the agent into rats with growing sarcomas had no visible effect upon their tumors.

Since the filterable agent shows no specific affinity for the neoplasm, and no immunological cross-relationship has been demonstrated between the two, the only conclusion which can be drawn at the present time is that the agent is but a chance contaminant of the sarcoma.

One is then confronted by the question: How did the filterable agent
first gain access to the tumor? To the obvious reply: From the host, there are several objections. If sarcoma 39 is being contaminated from time to time through propagation in a widely infected breed of rats, many of these animals should be immune to the agent, yet the rat has still to be found that is refractory to the usual dose. Secondly, it would be a curious coincidence indeed if the occasional infected growth had happened each time to be employed for incubation or other treatment during the investigations on tumor immunity, and never for routine transplantation. In the third place, we have seen no evidence that this filterable agent can be passed spontaneously from rat to rat, at least in an active state. And, finally, it shows no disposition to invade the neoplasm even after a relatively enormous amount has been deposited under the skin and it has reached the lymph nodes, suprarenal glands, spleen, and brain.

If, on the other hand, the sarcoma became infected once and for all at some time in the past, and the filterable agent is now established permanently therein, why has it never declared itself during the 20 years throughout which this tumor has been cultivated? And, since it induces a fairly high immunity, why are rats bearing the sarcoma susceptible to the agent, when Rivers and Pearce (7) found that Brown-Pearce carcinomas infected with virus III or vaccine virus immunized rabbits against these two viruses? The lack of resistance cannot be explained by the assumption that the filterable agent remains imprisoned within the tumor cells, and therefore non-antigenic, since all cells except those at the periphery of the growth undergo necrosis as it enlarges, and because even rats in which sarcoma 39 has been completely absorbed are as susceptible to this agent as any stock rat.

The supposition that upon its original encounter with this neoplasm the filterable agent was lying latent in a rat, plausible enough at first sight, loses all force with the realization that even when the filterable agent has been disseminated throughout the host's organism it has no propensity to invade the tumor.

There is no difficulty in dismissing at once any proposal that the filterable agent may have entered from some outside source, for in 25 years' experience during which hundreds of routine transplantations have been performed with this neoplasm, the characteristic infection has never appeared.
Thus no explanation can be advanced which will fit all the evidence, unless one is prepared to consider the assumption of a filterable agent with duplex capabilities, able to induce inflammation under some circumstances and neoplasia under others. The situation in such a case would resemble that described by Andrewes and Shope (8-10), in which there was encountered an aberrant inflammatory strain of the rabbit "fibroma" virus. Another variant of this virus has been recorded by Berry (11), and many other viruses are known to undergo qualitative changes in pathogenicity without alteration of immunogenic power, the variant strains continuing to cross-immunize. As there was no evidence of cross-immunity between agent and tumor in the present instance, a mutation seems improbable.

It is conceivable that the pyogenic filterable agent herein described is one already known but now masquerading under an unfamiliar form in the rat. The virus of infectious ectromelia (12) produces swollen feet, but in this disease the paws are edematous at first and later gangrenous, never frankly suppurative. Furthermore, the virus of ectromelia is passed by contact, elicits extensive necrosis in the liver and spleen, is associated with the presence of abundant inclusions and the production of neutralizing antibodies, and is not pathogenic for the rat, all of which sets it definitely apart from the present agent. A thorough investigation of its immunological relationships and other features will be required before the latter can be assigned a final position among the infectious diseases.

SUMMARY

A filterable agent resembling the viruses is described. It was encountered in sarcoma 39, a propagable neoplasm of the white rat, and has now been maintained in this species for 28 passages over a period of some 7 months without appreciable loss in virulence. Its chief effect is the production of large abscesses in an animal species comparatively resistant both to viral diseases and suppuration. The white mouse is more susceptible than the white rat, the rabbit less so, and the guinea pig highly resistant.

The agent has been repeatedly recovered from sarcoma 39 treated in special ways, but under the ordinary circumstances of routine
transplantation it does not manifest itself. As yet there is no certainty on where it came from or how it maintains itself under natural conditions.

We are indebted to Miss Hede J. Frank, who did much of the preliminary bacteriological work, to Miss Eleanor Molloy, who cultivated the agent in the egg, and to Dr. John G. Kidd, Dr. Thomas M. Rivers, and Dr. Albert B. Sabin, all of whom have given generously of their time to review our evidence and examine our material.

BIBLIOGRAPHY


EXPLANATION OF PLATE 17

Fig. 1. A 4 day abscess, showing the thick wall and the congestion in the adjoining subcutaneous tissue.

Fig. 2. An 11 day abscess, consisting of a thin walled pus sac. Below and to the left a smaller abscess is seen.

Fig. 3. Suppurative lesions of the forepaws in a rat inoculated 14 days previously in the testis.

Fig. 4. Necrosis and polymorphonuclear infiltration 24 hours after intradermal inoculation. At the lower edge of the photograph appears the margin of a large abscess. Hematoxylin and eosin. ×150.

Fig. 5. Cytoplasmic inclusion in epidermis overlying a 1 day intradermal abscess. Eosin and methylene blue. ×600.

Fig. 6. Cytoplasmic inclusion in epidermis overlying a 1 day intradermal abscess. Eosin and methylene blue. ×1600.