EXPERIMENTS ON THE PRODUCTION OF SPECIFIC ANTISERA FOR INFECTIONS OF UNKNOWN CAUSE.

II. The Production of a Serum Effective against the Agent Causing a Chicken Sarcoma.

By PEYTON ROUS, M.D., OSWALD H. ROBERTSON, M.D., and JEAN OLIVER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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The work detailed in Part I of this paper has served to demonstrate the theoretical usefulness of exhausted sera to combat infections, but it presents no instance of a serum actually resulting from the direct immunization of animals by injections of infected tissue. Such an instance is highly desirable. It has been furnished through experiments with a transplantable chicken sarcoma,[1] known in our laboratory as Chicken Tumor I, which has a filterable agent as its cause. The exact nature of the filterable agent is unknown, but its general characters would seem to place it with the microorganisms.[2] The tumor is a typical sarcoma, highly malignant, and as a rule rapidly fatal to fowls developing it after an implantation with neoplastic tissue or inoculation with the Berkefeld filtrate of a tumor suspension. Some individuals are primarily insusceptible, and in some the growth develops slowly, and eventually retrogresses. The latter fail ordinarily to develop a sarcoma when reinoculated. Repeated unsuccessful attempts have been made to demonstrate antibodies in the blood of fowls in which a growth has retrogressed, and to render others immune to the tumor by injections with heated or dried neoplastic tissue.[3] The tumor cannot be transmitted to geese, ducks, pigeons, or mammals; but attempts to develop an antiserum by the immunization of such animals have been blocked through failure to obtain the tumor-

[1] Rous, P., *J. Exp. Med.*, 1910, xii, 696; *J. Am. Med. Assn.*, 1911, lvi, 198.

[2] Rous, P., and Murphy, Jas. B., *J. Am. Med. Assn.*, 1912, lviii, 1938.

[3] Rous, P., and Murphy, Jas. B., *J. Exp. Med.*, 1914, xx, 419.

producing agent in culture. The employment in these alien species of the neoplastic tissue itself as an antigen, or a filtrate from such tissue, elicits, of course, anti-chicken elements in the immunized individual.[4] The method of specific absorption to obtain an antiserum here finds a direct application.

Immunization of Animals.—The blood of fowls carrying the chicken tumor often contains during the last few days of life the causative agent of the disease; and in the sarcomatous tissue the agent is regularly present in large quantity. Both blood and tissue could therefore be used in the immunization, which was desirable in order to insure the production of a strong anti-chicken serum. Chickens moribund with the growth were bled to death under aseptic conditions, the blood was citrated, and the tumor tissue itself was ground with sand and suspended in Locke's solution just prior to injection. As the causative agent of the growth will withstand repeated freezing and thawing and retains its activity for a long period at low temperature, the material often was kept in the frozen state for days or weeks prior to use.

The first attempts to obtain an antiserum were made with rabbits. A number of these animals were injected intravenously on 3 successive days with a tumor extract in salt solution, and thereafter intraperitoneally every 6 days with citrated chicken blood and a suspension of tumor tissue. But though the serum soon acquired a high content of chicken hemolysins and hemagglutinins it had not the least neutralizing effect on the tumor-causing agent present in Berkefeld filtrates of suspensions of the sarcoma tissue. For this reason work with rabbits was at length discontinued.

Implanted bits of the chicken sarcoma perish at once in mammals, whereas in ducks and pigeons they grow for some days before retrogressing and may form quite large nodules. It seemed from this fact not improbable that birds would prove more favorable than rabbits as producers of tumor antibodies, owing to what might be considered as a partial susceptibility on their part to the neoplastic disease. For Flexner and his associates[5] have shown that in the case of poliomyelitis an immune serum is obtained only in species susceptible to the infection. Geese were used, therefore, in the further attempts to obtain an antiserum Their immunization was carried out as follows:

Goose A received three intravenous injections on successive days of mixed tumor suspension and citrated blood from fowls moribund of the growth, followed thereafter every 6 or 7 days by intraperitoneal injections of the same material. Goose B was given the same sort of material, but only into the peritoneal cavity. From time to time both birds were bled from a wing vein and the sera compared

[4] Bailey encountered this difficulty in experiments on complement fixation with the serum of pigeons inoculated with the growth (Bailey, C. H., *Med. Rec.*, 1915, lxxxviii, 403).

[5] Personal communication from Dr. Flexner.

in neutralizing properties with those of three normal geese. During a long period of immunization no differences could be noted, but on the contrary a similarity in the five sera so entire as to indicate that the blood of one normal goose is just like that of any other in its effect, or rather lack of effect, on the tumor-producing agent. At length, as will be seen from the experiments now to be cited, an immune principle became demonstrable in the serum of the injected birds.

Experiment 1.—The immunized geese, A and B, were bled for serum 84 and 66 days respectively after immunization was begun, and 9 days after the last injection of tumor material. Goose A had received three intravenous and ten intraperitoneal injections, while Goose B had but nine injections, all intraperitoneal. Two normal geese (a and b) were bled at the same time and to an equal amount; namely, 75 cc. The sera were inactivated as usual, and all were submitted to absorption with similar portions of washed chicken red cells, as follows:

Mixture.	Hemagglutination.	
	Immune sera.	Normal sera.
25 cc. of goose serum + 5.2 cc. of chicken red blood cells incubated 1 hr. and	+	0
serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr.	Tr.	0

Cultures taken after the second absorption proved sterile. Preliminary tests showed that the untreated immune serum failed to hemolyze chicken cells when chicken serum was used as complement, whereas these were rapidly destroyed in the presence of guinea pig complement. Consequently the latter was used in the titrations that follow.

Anti-Chicken Titer of the Sera. Hemolysis.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent guinea pig red cells. Incubation and ice box was for 2 hrs. at 38°C. and readings were made after the tubes had stood in the ice box over night.

Serum.	Serum dilution.								Complement + red cells + 0.2 cc. of salt solution.
	0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	
Untreated immune. A..	C.	C.	C.	Alm. C.	Alm. C.	+++	Tr.	Ft. Tr.	0
Untreated immune. B..	++++	+++	++	+	+−	Tr.	Ft. Tr.	0	
Untreated normal. a...	Tr.	Ft. Tr.	0	0	0	0			
Untreated normal. b...	"	" "	Ft. Tr.	0	0	0			
Exhausted sera...	No hemolysis by any.								

The selective absorption had completely deprived the immune sera of their relatively strong hemolysin.

Hemagglutination.—This was read in mixtures similar to the foregoing but containing chicken serum (1 in 10) as complement. None of the tubes showed any hemolysis with this complement, but those containing undiluted immune goose serum exhibited a slight hemagglutination. None of the exhausted sera agglutinated chicken cells in the least.

Precipitation.—The normal sera contained no precipitin, but a weak one was present in the immune sera. It was active against dilutions of chicken serum up to, and including, 1 in 40.

In Vivo Tests of Neutralization.—The exhausted sera only were used in neutralization tests. For this purpose mixtures were made of the sera with a Berkefeld filtrate containing the tumor-producing agent, and these after incubation were injected into fowls. In some early experiments mixtures of the filtrate with isotonic saline or Locke's solution were employed as controls, but it was found that they soon lost their tumor-producing activity when incubated, whereas this was retained in mixtures with normal goose serum, either untreated or exhausted. Consequently in the present experiment, as in others to be detailed, the mixtures with normal sera constitute the controls.

The tumor filtrate was prepared by grinding fresh neoplastic tissue with sand, making a thin suspension in Locke's solution, shaking, centrifuging, and passing the clear fluid through one or another of several Berkefeld filters (N). Several filters were used to ensure an active filtrate, since the tumor-producing agent is held back by many of the finer Berkefeld candles, and all the filtrates were united. Now two mixtures were made with the sera: (1) 15 cc. of each exhausted serum + 7 cc. of filtrate; (2) 7 cc. of each exhausted serum + 2 cc. of filtrate. These were incubated for 2 hours at 38°C. They remained water-clear. 1 cc. of a suspension of sterile diatomaceous earth was added to each, and portions of all were injected into each of a number of chickens. The mixtures with immune sera were injected first so that any possible advantage as regards attenuation of the virus during incubation, or neutralization of it, might lie with the mixtures containing the normal serum. Diatomaceous earth was added because, through the tissue injury it causes, the production of tumors by a filtrate is rendered much more certain.[6]

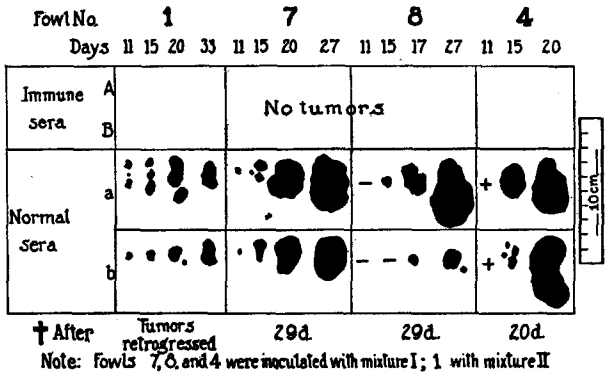
The ten chickens inoculated received 3 cc. of each mixture, into the pectoral muscles and the muscles of the upper wings respectively. Usually the tumor grows fastest and becomes largest in the pectoral muscles, and for this reason the injection site for the mixtures was varied from bird to bird; but in the experiment now under consideration no favoring influence of the pectoral situation

[6] Rous, P., Murphy, Jas. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1751.

was to be seen. The growths did not attain a very large size before death ensued from metastases.

Clear-cut findings were obtained, as Text-fig. 1 shows. Only four of the ten fowls developed tumors. In them growths failed to appear where the mixtures of immune sera and filtrate had been injected, whereas at the control sites large ones developed.

Experiment 2.—The same general plan was followed as in the preceding experiment, but the immunized geese had now received two additional intraperitoneal injections. Bleeding for serum was done 121 and 103 days respectively from the time immunization of the birds was started, and 7 days after the last



TEXT-FIG. 1. The tumors in four fowls receiving intramuscular injections of mixtures of tumor filtrate with immune and normal goose sera respectively.

injection. The sera of three normal geese, a, b, and c, were used in control. Selective absorption was carried out as usual.

30 cc. of goose serum + 5.8 cc. of chicken red cells incubated 1 hr. and
serum transferred to 2.9 " " " " " " 1 " "
" " " 2.8 " " " " " " 1 " "

Cultures taken after the last absorption proved sterile.

Anti-Chicken Titer of the Sera. Hemolysis.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent chicken red cells.

Serum.		Serum dilution.								
		0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Untreated immune.	A.....	C.	C.	C.	Alm. C.	++	+	Tr.	0(?)	0
	B.....	Alm. C.	Alm. C.	+++	++	+	Ft. Tr.	0	0	0
Untreated normal.	a.....	No hemolysis.								
	b.....	+−	0	0	0	0				
	c.....	+++	++	+	0					
Exhausted sera.....		No hemolysis by any.								

Hemagglutination.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 5 per cent chicken red cells + 0.2 cc. of salt solution.

Serum.		Serum dilution.				
		0	1/2	1/4	1/8	1/16
Untreated immune.	A.....	+	+	+	Tr.	0
	B.....	++	Tr.	0	0	0

With the exhausted normal and immune sera, as well as the untreated normal sera, no agglutination was obtained.

Precipitation.—There was no precipitin in the normal sera, but one was present in that from both immune geese. It was effective in mixtures of equal parts of the undiluted goose serum with dilutions of chicken serum up to and including 1 in 40 for Goose A and 1 in 20 for Goose B. The titer was little if at all diminished by the absorption with red cells.

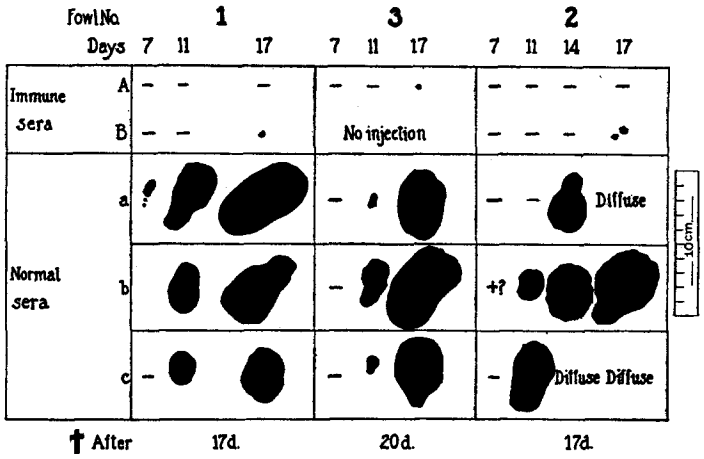
In Vivo Tests of Neutralization.—A Berkefeld filtrate of a tumor extract was prepared by the method already described, and three mixtures were made of it with the exhausted sera, both normal and immune.

Proportion X: 7.5 cc. of serum + 2 cc. of filtrate.
Proportion Y: 12 cc. of serum + 6 cc. of filtrate.
Proportion Z: 7 cc. of serum + 7 cc. of filtrate.

Incubation was for 2 hours at 37°C. No precipitation or clouding occurred. A suspension of diatomaceous earth was now added to each mixture in the amount of one-tenth its volume, and the injection of fowls was forthwith begun. Fifteen fowls were used, and all save four received 3 cc. of each mixture, the site of injection being varied. The four fowls mentioned were not given the mixture con-

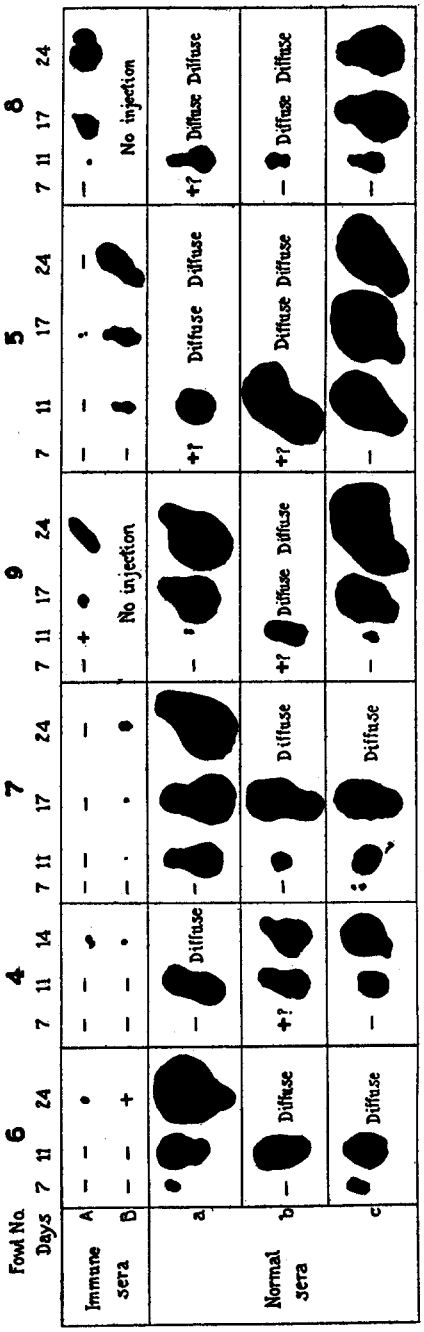
taining the serum of Immune Goose B. The injections were made into the upper wing, upper leg, and pectoral muscles. As Text-figs. 2, 3, and 4 show, large growths rapidly developed where the control mixtures had been placed, whereas none, or only slowly growing ones, were caused by the mixtures containing immune serum.

The neutralizing effect on the tumor-producing agent of the exhausted serum of geese immunized with tumor tissue is clearly shown by these protocols. The agent was especially active in the filtrate used in Experiment 2, as shown by the fact that every one of the fifteen inoculated fowls developed tumors—an occurrence unparalleled in our records. The immune serum completely prevented

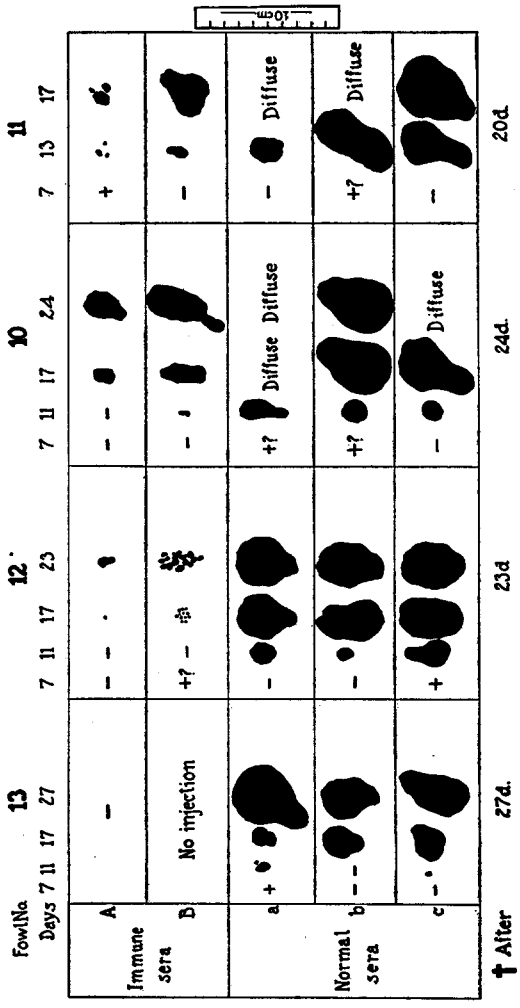


TEXT-FIG. 2. The tumors developing in three fowls receiving mixtures in Proportion X.

tumors at only three injection sites in these fowls, though its protective influence was manifest wherever it had been injected. Very large tumors resulted from all three normal serum mixtures, whence it may be inferred that even the smallest amount of filtrate present in any one, namely that of Proportion X (about 0.66 cc. of filtrate per fowl), contained what might be termed a maximum tumor-producing dose of causative agent. More than twice this amount (1.5 cc. in Proportion Z) yielded tumors that were no larger and grew no more rapidly. The test of the neutralizing power of the immune sera was evidently a severe one in this experiment. In Experiment 1 the filtrate was far less active, as shown by the large proportion of nega-



TEXT-FIG. 3. Tumors in six fowls receiving mixtures in Proportion V.



TEXT-FIG. 4. Tumors in four fowls receiving mixtures in Proportion Z.

tive fowls (six out of the ten inoculated) and the slow course of the tumors that appeared. Here the neutralization of the tumor-producing agent by the exhausted serum of the immunized geese was complete.

To what is the neutralization referable,—unabsorbed remnants of chicken antibodies? This possibility may be tested by determining whether chicken antibodies as such are able to neutralize the tumor-producing agent. The results with the sera of immunized rabbits gain importance in this connection. For the rabbit sera, while strongly anti-chicken—many times more so than the goose sera—had not the least neutralizing effect on a tumor filtrate.

Experiment 3.—A rabbit was given three intravenous injections on successive days of a saline extract of chicken tumor, followed at 6 day intervals by eight intraperitoneal inoculations of a mixture of tumor suspension and citrated blood from fowls moribund of the growth. 8 days after the last injection the animal was bled to death, and its inactivated serum was compared in neutralizing power with that of a normal rabbit. Selective absorption of both was carried out as usual.

Mixture.	Hemagglutination.
15.5 cc. of rabbit serum + 4 cc. of chicken red blood cells, incubated 1 hr. and	Marked.
serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr.....	0

Anti-Chicken Titer of the Sera. Hemolysis.—0.25 cc. of inactivated serum in graded dilutions + 0.25 cc. of 1 in 10 guinea pig complement + 0.25 cc. of chicken red cells.

Immune serum.	Serum dilution.															Guinea pig complement + salt solution + red cells.
	0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1,024	1/2,048	1/4,096	1/8,192	1/16,384	
Untreated..	C.	C.	C.	C.	C.	C.(?)	Alm. C.	+++	+++	+++	+	±	Tr.	Tr.	Ft. Tr.	0
Exhausted..	+	+	±	±	Tr.	Tr.	Tr.	F. test.	0	0						

Exhaustion was in this instance only approximately complete.

Hemagglutination.—The mixtures were the same as those above except that 0.25 cc. of 0.9 per cent salt solution was substituted for guinea pig complement.

Immune serum.	Serum dilution.									
	0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Untreated.....	+++	Alm. C.	Alm. C.	C.	C.	++	++	Tr.	—	0
Exhausted....	No agglutination.									

The normal rabbit serum destined to be used in control of the *in vivo* work caused only the slightest hemolysis of chicken cells and no agglutination, when tested prior to its absorption. Thereafter it did not affect the cells at all.

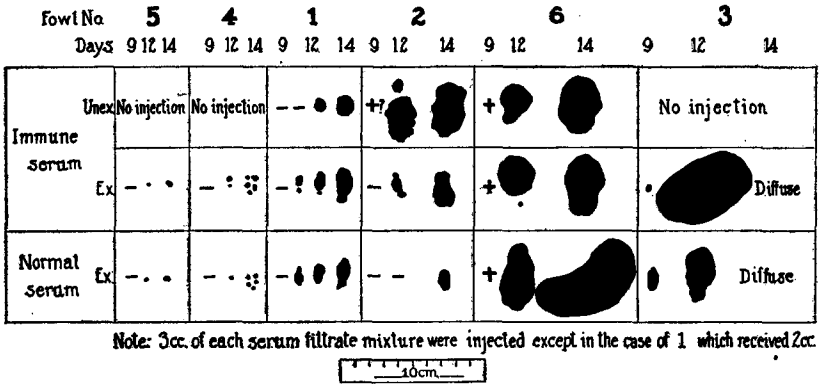
Precipitation.—The normal rabbit serum was entirely inactive, but that of the immunized animal caused precipitation when incubated with equal parts of chicken serum diluted up to and including 1 in 2,560.

In Vivo Tests of Neutralization.—Three serum specimens were used—normal and immune serum, exhausted as above, and untreated immune serum. A Berkefeld filtrate containing the tumor-producing agent was prepared as usual and mixed with the rabbit sera in the proportion of 6 cc. of filtrate to 12 cc. of serum. Incubation at 38°C. was carried on for 2 hours, cultures were taken, portions of a suspension of diatomaceous earth in salt solution were added to each mixture (0.7 cc. for every 20 cc. of mixture), and injections were made of 3 cc. into five fowls and of 2 cc. into a sixth. In the mixtures with immune serum a floccular precipitate had come down which was distributed by shaking prior to the injections. The sites of injection were varied, as usual. The cultures of the injection fluids were negative after 2 days. Tumors developed in all the fowls, as Text-fig. 5 shows.

The test of the neutralizing power of the rabbit sera was in this case not a severe one. For the late appearance and slow growth of the control tumors clearly showed that no excess of tumor-producing agent was present in the mixtures. Yet there is not the slightest indication of any effect upon the agent of the immune serum, even when it had not been exhausted and was very strong in chicken hemolysin, agglutinin, and precipitin. Said serum had exactly the same effect as serum from a normal rabbit, which contained only the weakest antibodies for the chicken. A floccular precipitation occurred in the mixtures of filtrate and immune serum, but so slowly that it can scarcely have afforded to the tumor-producing agent much protec-

tion from other serum antibodies; and only complete protection by it would explain the results in the inoculated fowls.

This experiment would seem to prove that the neutralization of the tumor-producing agent by the serum of immunized geese is not due to antibodies directed against chicken tissue as such. Such antibodies —or at least those elicited in the immunization of rabbits—fail entirely to injure the tumor-producing agent, even when they are very strong. In view of these facts, the conclusion seems justified that the neutralization of the agent causing a chicken tumor by the serum



TEXT-FIG. 5. Tumors arising in six fowls injected with tumor filtrate mixed with normal and immune rabbit serum.

of geese repeatedly injected with the tumor tissue is not the result of the action of antibodies directed against the chicken tissue as such, but is due to others specific for the tumor-producing agent. These are retained by goose serum exhausted with chicken red cells.

DISCUSSION.

The selective absorption of tissue antibodies has been applied thus far to four immune sera of widely different properties (see Part I of this paper), with success in each instance. There is no doubt that by the method sera can be deprived of antibodies immediately injurious to the animal organism while retaining those directed against an infectious agent or its products. Applications of the principle in the treatment of disease at once suggest themselves. But many points

must be determined before any practical therapeutic venture is warranted.

First, the late or latent effects on the animal body of exhausted serum must be closely studied. Serum precipitins are not removed with hemolysins and hemagglutinins during the process of exhaustion with red cells. What then is the effect of a specific precipitin acting *in vivo* on an animal of the species against which it is directed? We have been unable to find in the literature a conclusive answer to this obvious question. The controversy over the relation of precipitation to anaphylaxis has resulted in a multitude of *in vivo* experiments, but these have been carried out almost exclusively by introducing precipitin and precipitinogen into animals to which both are alien, or by injecting a serum precipitinogen into an organism that possesses, or will develop, a precipitin for it. Uhlenhuth and Haendel[7] and Doerr and Moldovan[8] have claimed that anti-guinea pig rabbit serum of high precipitin titer is toxic to guinea pigs when injected intravenously; but these authors made no attempt to absorb from the serum the hemolysins and agglutinins present in it and undoubtedly capable of harmful effects. Their work has not been followed up. We plan to do this.

It seems not unlikely that an antiserum resulting from injections of tissues, especially tissues other than blood, will contain elements of possible harm besides hemolysins, hemagglutinins, and precipitins. Here one is confronted with the problem of the specificity of cytotoxins, so long and indecisively debated. Fortunately we are concerned with a single aspect of this problem; namely, that of whether specific cytotoxins, assuming that they exist for the generality of organs—a large assumption—can be removed from serum by its exhaustion with red corpuscles. For should they not be so removable it may be necessary to exhaust a serum with the same kind of tissue employed in the immunization, a matter of much practical difficulty. Experiments on the point with a specific cytotoxic serum, so called, have been begun.

Theoretically the most important use of exhausted sera lies in the treatment of infections of unknown cause. And with each such in-

[7] Uhlenhuth and Haendel, *Z. Immunitätsforsch., Orig.*, 1910, iv, 761.

[8] Doerr, R., and Moldovan, J., *Z. Immunitätsforsch., Orig.*, 1910, vii, 223.

fection two fundamental points would have of necessity to be determined. They are (1) whether the infected tissue will suffice as a practical antigen, and (2) whether the antibodies useful against the infection or its products will survive the serum's exhaustion of antibodies injurious for tissue. The microorganisms in infected tissue employed as antigen will be in many instances in the highest state of pathogenicity. There are advantages to this, but also drawbacks. If the animals to be immunized are themselves susceptible to the infection much less fresh tissue antigen can be employed than of one attenuated by culture or in another way. The dosage of antigen will also be difficult to regulate. Both these obstacles were encountered in Part I of the present work, during our attempts to immunize dogs by injecting them with the blood of rabbits dying of pneumococcus septicemia. So large a percentage of the dogs died that resort was had at length to an antigen of normal tissues and pneumococcus cultures injected separately. The conditions would be much more favorable to successful immunization in the case of infections only slightly pathogenic to the animals employed for immunization. Here tissue containing the infective agent in most virulent form would have great advantages and not improbably decisive ones in the case of cultivable agents that lose their pathogenicity, and incidentally their usefulness as antigen, when grown *in vitro*. Furthermore, it is conceivable that with an agent in highly virulent form so little of the tissue containing it might in certain instances be required as antigen that the serum's titer in elements injurious for tissue would be slight, and the exhaustion in consequence a relatively simple matter.

Little can at this time be said on the persistence of desirable antibodies in an exhausted serum, further than that our experiments make this seem probable in most instances, as do also the observations of others who have used the method of selective absorption to a different end; namely, to demonstrate the specificity of antibodies.[9] Should it become necessary to exhaust a serum of precipitin by means of precipitation in order to render it harmless *in vivo*, even this, it

[9] A noteworthy demonstration of the possibilities of the method is to be found in the work of Todd, C., and White, R. G., *Proc. Roy. Soc. London, Series B*, 1910, lxxxii, 416. By the selective absorption of induced isohemolysins these authors were enabled to recognize the red corpuscles of individual oxen.

would seem, might be done without, in most instances, removing the antibodies directed against an infectious agent. For Gay and Stone[10] have made many attempts to bring down such elements in a serum precipitate, but without success.

Although the use of exhausted serum in the treatment of infectious diseases is at present but a distant possibility, there lies open a field for its immediate employment. Through the method of absorption much may be learnt regarding serum immunity to animal diseases—as witness the case of the chicken sarcoma,—and to human infections of unknown cause that are transmissible to animals. For the tissues of infected animals will furnish a ready antigen for experimental purposes, while normal individuals of the same species can be used as test objects to determine whether the exhausted sera resulting from immunization possess any protective power. A concrete illustration of such a possibility is afforded by some recent work of Nicolle and Blaizot.[11] These authors state that they have produced an effective antityphus serum in donkeys by injection with the spleens of guinea pigs dying of the disease. The serum is intended for use in human beings, but they find that with it guinea pigs can be cured of typhus, though the serum is so toxic for such animals that it can be given only in small quantities, which hinders the tests. It would have been interesting to deprive the serum of this toxicity by selective absorption with guinea pig cells, with a view to a more striking demonstration of its antityphus power.

SUMMARY.

By the method of selective absorption with tissue, protective serum antibodies have been demonstrated in the case of an infection of unknown cause; namely, a chicken sarcoma transmitted by a filterable agent. Geese were repeatedly injected with the finely ground sarcoma and with blood from fowls moribund of it; and their sera acquired the power to prevent the tumor-producing agent from causing growths. That this was not due to antibodies elicited by the chicken tissue as such was shown by exhaustion of the goose sera with chicken

[10] Gay, F. P., and Stone, R. L., *J. Immunol.*, 1916, i, 83.

[11] Nicolle, C., and Blaizot, L., *Ann. Inst. Pasteur*, 1916, xxx, 446.

red cells, a step which had not the least effect on the tumor-preventing power, and also by experiments with rabbits immunized as were the geese. These animals developed strong chicken antibodies in their sera which failed nevertheless to affect the tumor-producing agent.

Serum immunity to the chicken sarcoma is weak at best; and in the case of some other infections of unknown cause, more striking results may be anticipated from the method of selective absorption. It is even conceivable that by its means sera of therapeutic usefulness may become available. But much remains to be settled as regards the dangers of exhausted sera and the limitations of the method. Fortunately there exists an immediate field for the latter in laboratory studies on the nature of immunity to infections of which the cause has not been recognized.