STUDIES ON HETEROPHILE ANTIGEN AND ANTIBODY.*

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III.—On Heterophile Anaphylaxis.

It has been shown by Friedberger and his associates, Hartoch (1909^{15, 20}) and Castelli (1910¹⁴), that the sera of rabbits, obtained after the injection of sheep's blood corpuscles or serum, are powerfully toxic for guinea-pigs, and on intravenous injection cause death acutely. This has been confirmed by numerous investigators, and a number of new facts have been ascertained. The clinical symptoms and post-mortem appearances of animals treated in this way have been generally recognised as closely similar to those of guinea-pigs which have suffered from anaphylactic shock (Friedberger (1909¹³) with Castelli (1910¹⁴), Doerr and Moldovan (1910⁵), Graetz (1911¹⁹), etc.); and this phenomenon has been termed by Friedberger *antiserum anaphylaxis*, in contrast to antigen-antibody anaphylaxis in the true sense. Atropin prevents the shock to a certain extent (Friedberger and Castelli, Doerr and Moldovan, Mita (1911²³)). Intense reduction in complement accompanies the symptoms of intoxication (Friedberger and Hartoch); and the animals which survive are refractory to further injections of the toxic antisera, *i.e.*, they exhibit anti-anaphylaxis (Friedberger, Doerr and Moldovan). The toxicity of the antisera was reduced when they were injected along with the antigen, or when the antigen was injected beforehand (Friedberger and Castelli). Subcutaneous injection of the toxic antisera into guinea-pigs caused necrosis (Doerr and Moldovan). Thus there are many indications that the primary toxicity of anti-sheep immune sera from the rabbit for certain animals is of the nature of an anaphylactic reaction. It has also been shown by Friedberger and Castelli that after every repetition of the injection of antigen, the toxicity of the serum of the immunised rabbit is reduced for a time ("negative phase"), but rapidly reaches a maximum again, and then, in the absence of further injections, diminishes slowly. The antisera are deprived of their toxicity by digestion with the homologous antigen (Friedberger and Castelli, Doerr and Moldovan); thus

* Received September 20, 1921.

hæmolytic immune sera obtained by the injection of sheep corpuscles into the rabbit are detoxicated by treatment with sheep corpuscles, but treatment with guinea-pig's or horse's corpuscles has practically no effect in reducing the toxicity. Inactivation of the toxic sera at 56° C. has little effect in reducing their toxicity, but heating the antisera at 65° C. reduces the toxicity to a great extent (Friedberger and Goretti (1914¹⁶)). Thus there is also evidence that the toxic constituent of the antisera has the character of an antibody. But the difficulty in explaining the phenomenon as due to anaphylaxis lay in the fact that the toxicity of the antisera was not parallel to their content in any known antibody such as hæmolysin or precipitin. The primary toxicity of anti-sheep immune sera from the rabbit appeared also to be peculiar, because the majority of antisera from the rabbit obtained by the injection of blood corpuscles or sera from other animals have been shown to be practically non-toxic to guinea-pigs. A certain degree of toxicity has, however, been recognised in some specimens of antisera after multiple injections of large amounts of antigen (Doerr and Moldovan, Biedl and Kraus (1910³), Kraus and Mueller (1911²¹), Graetz, etc.). But, in the case of anti-sheep immune sera from the rabbit, toxicity is the rule, and minimal amounts are lethal in a few minutes (the lethal dose per 100-gram weight of guinea-pig = 0.03 c.c., Doerr and Weinfurter (1912⁸)), even when the serum has been obtained after a single injection of antigen. Such immune sera from the rabbit are not the only antisera which exhibit high primary toxicity for guinea-pigs, since it has been shown by Friedberger and Mita (1911¹⁷) and others that those obtained after the injection of horse serum, typhoid bacilli, or brewers' yeast into rabbits were strongly toxic. It is striking that the species of the animal from which the antiserum has been derived is one of the important factors, since antiserum derived from the guinea-pig after the injection of sheep corpuscles has been shown to be non-toxic. Moreover, the development of the toxic effect depends on the species of animal which receives the injection of antiserum, *e.g.*, the anti-sheep corpuscle immune serum from the rabbit, which is strongly toxic for guinea-pigs, is practically non-toxic for normal rabbits, even five to six minimum lethal doses for the guinea-pig having been injected without effect (Doerr and Moldovan). The facts are summarised in Table I.

TABLE I.

Animal yielding Antiserum <i>v.</i> Sheep Corpuscles.	Effect upon	
	Guinea-pig.	Rabbit.
Rabbit	toxic	non-toxic
Guinea-pig	non-toxic	non-toxic

A number of hypotheses have been offered to explain the primary toxicity of such special antisera. Among these, Friedberger and his associates¹⁴ have suggested an ingenious explanation, viz., that the toxic antisera contain a residuum of antigen, in addition to the antibody, but that these constituents do not react with each other in the rabbit's body, perhaps for the reason that the antigen has undergone some alteration. It is only when the material is introduced into the guinea-pig that a specific reaction occurs with the intervention of complement, thus causing the development of anaphylactic shock in the host. This view has been strongly opposed by v. Dungern and Hirschfeld (1911⁹), Doerr and Weinfurter, and others. None of the hypotheses has been generally accepted as explaining this mysterious phenomenon of primary toxicity. There is no close zoological relationship between guinea-pig and sheep, and a difficulty stood in the way of including this phenomenon in the anaphylactic group due to interaction between protein antigen and antibody, because there is no appreciable antibody in the toxic antisera for the serum or blood corpuscles of the guinea-pig. The first light was thrown on this problem by Forssman (1911¹¹), who demonstrated the existence of the same "receptor" in sheep corpuscles and guinea-pig's tissue, whereby guinea-pig's organs can generate powerful hæmolytic immune body for sheep corpuscles when injected into the rabbit ("heterophile immune body"). Soon after, along with Hintze (1912¹²), he showed that not only does the serum of rabbits, thus immunised, actively hæmolyse sheep corpuscles, but if injected intravenously into the guinea-pig, there results intoxication similar to the antiserum anaphylaxis of guinea-pigs. This is spoken of as "heterophile toxicity," *i.e.*, the toxicity exhibited by a serum which contains heterophile antibody towards an animal whose tissues contain heterophile antigen. The phenomenon has been examined by a number of investigators both on account of the interesting relationship which it bears to the question of specificity of immunity reactions (Orudschiew (1913²⁵), Tsuneoka (1914³⁴), and others), and also with a view to finding an explanation of the primary toxicity of such special antisera (Forssman and Hintze, Doerr and Pick (1913^{6, 7}), Amako (1914¹), Sachs and Nathan (1913²⁸), etc.). In addition to the tissues of the guinea-pig, a number of animal tissues have been found to possess the capacity to generate heterophile immune body for sheep corpuscles when injected into suitable animals, *e.g.*, organs of horse, dog, cat, mouse, fowl, tortoise, and several kinds of fish (spoken of as animals of "guinea-pig type"), although this property is not exhibited by organs of many other closely related animal species, *e.g.*, ox, rabbit, pig, man, rat, goose, pigeon, frog, and eel (animals of "rabbit type"). It is also absent from the blood corpuscles of all the animals mentioned, but it is present in the blood corpuscles of the sheep and goat, whose organs do not possess this property. The antigen has further been found in paratyphoid and Gaertner bacilli,

mouse tumour, sheep spermatozoa and egg yolk of the fowl. The curious distribution of heterophile antigen in the different species of animals, without any obvious relationship to species specificity or to the group reactions of related species, and thus in contrast to the general rule in the case of immunity reactions of proteins, has been explained rationally, since it has been shown by the writer (1920³¹, 1921³³) and others that the specificity of heterophile antigens resides in their lipoidal constituents.

The primary toxicity to particular animals of the antiserum from the rabbit *v.* sheep corpuscles should be included among the heterophile characters of such antisera, as it is known that sheep corpuscles contain heterophile antigen and possess the capacity to generate heterophile antibody when injected into the rabbit (Doerr and Pick,⁶ Sachs and Nathan). Thus a number of striking phenomena concerned with such primary toxicity, which had been observed before the discovery of heterophile antigen, have been explained by the results obtained from the investigation of the heterophile toxicity of antisera. It has also been found that the sera of the animals of guinea-pig type contain heterophile antigen (Orudschiew and others), although the power of such sera to generate heterophile antibody is far weaker than that of tissues from the same animal. Accordingly, the heterophile toxicity of certain antisera obtained by injecting sera into suitable animals could also be anticipated.

As will be seen, there are strong reasons for supposing that the toxic molecule of heterophile antiserum, obtained from the rabbit by the injection of heterophile antigens, is identical with the heterophile antibody. Thus the mechanism of the intoxication produced by an injection of the antiserum into suitable animals may be supposed to be anaphylactic, the antigen present in the animal's tissues reacting with the antibody contained in the injected antiserum, *i.e.*, there is *anaphylaxie inverse*, as it is termed by Turró and Gonzalez (1912³⁵). The following results form the basis of this view: (1) antisera containing heterophile antibody, in general, cause anaphylactic shock when injected into suitable animals (heterophile antisera from rabbit *v.* organs of guinea-pig, horse, dog, cat, fowl, and tortoise have been examined); (2) heterophile antisera are toxic only to animals which contain heterophile antigen in their tissues, but are not toxic to animals which do not contain the suitable antigen; (3) the toxicity of heterophile antisera is removed by contact with heterophile antigens, but not with non-heterophile tissues; (4) organs of animals which do not contain heterophile antigen (tissues of ox, rabbit, rat, pig, man, pigeon, etc.) lack the power to generate toxic antisera; (5) it is known that animals of guinea-pig type are incapable of generating heterophile immune body, and such animals also fail to produce toxic antisera when injected with heterophile antigens.

The writer³³ has demonstrated the property of heterophile antisera

whereby they fix complement specifically along with lipoids of heterophile antigens. Specific precipitate formation with the same combinations has also been observed by Sordelli and Pico (1919²⁶), and by Sachs and Guth (1920²⁷). Experiments on the qualitative and quantitative relationships between the complement-fixing and precipitating properties of heterophile antisera and their content in heterophile hæmolysin for sheep corpuscles have indicated that all the effects are due to the same antibody molecules (Taniguchi (1921³³)). These latter results also strongly support the view that the toxic character of such antisera is dependent on the heterophile antibody which they contain. However, it has been generally recognised (Friedberger and Castelli, Forssman and Hintze, etc.) that in the case of antisera obtained from the rabbit by injecting fresh sheep corpuscles, the primary toxicity for guinea-pigs is not quantitatively parallel to the content in hæmolytic immune body for sheep corpuscles. It has also been stated by Forssman and Hintze, and by Doerr and Pick, that in the case of heterophile antisera from the rabbit *v.* organs of guinea-pig, horse, or fowl, the intensity of the toxic effect produced in suitable animals is not always parallel to the content in hæmolytic immune body for sheep corpuscles. Therefore, Forssman and Hintze assumed that another antibody, different from the hæmolytic immune body, was responsible for the heterophile toxicity of the antisera. According to Sachs and Nathan, however, the primary toxicity for guinea-pigs of antisera obtained by injection of boiled sheep corpuscles is parallel to the content in hæmolytic immune body for sheep corpuscles; thus they believe that there is no necessity to assume multiple antibodies. This problem has been dealt with in the first place.

THE QUANTITATIVE RELATIONSHIP BETWEEN THE HETEROPHILE TOXICITY OF ANTISERUM AND ITS CONTENT IN HETEROPHILE ANTIBODY.

The amount of heterophile antibody contained in the different antisera was compared quantitatively by the intensity of their reaction with lipoids of heterophile antigens, *viz.*, complement-fixation and precipitation (see Taniguchi³³). In the case of heterophile antisera obtained by injecting heterophile organ antigens into suitable animals, such as the antiserum from the rabbit *v.* guinea-pig's organs, the hæmolytic power of the heated serum for sheep corpuscles also affords an index of the content of heterophile antibody. But in the case of antisera from the rabbit *v.* sheep corpuscles, the circumstances are different. Such antisera contain, at least, two different classes of sheep hæmolysin, one of which possesses heterophile character and the other is isophile (see Taniguchi³³). As has been demonstrated by the author, the amounts of the two different kinds of sheep hæmolysin

contained in different specimens of antiserum vary in their relative proportions, depending upon the method of immunisation and also upon the individuality of the animal from which the antiserum is obtained. As determined by the absorption of heterophile immune body with watery suspensions of heterophile tissues, sheep hæmolysin of heterophile type is relatively much more abundant in the serum after a single injection than after multiple injections (see Table II).

TABLE II.

Ratio in per cent. of heterophile and isophile hæmolysin content of antisera from the rabbit *v.* sheep corpuscles.

	Heterophile.	Isophile.	Number of Rabbits examined.
After one injection .	95-98	2-5	3
After three injections .	40-70	30-60	4

Accordingly, the results of experiments by Friedberger and Castelli, Doerr and Weinfurter, Forssman and Hintze, and others, showing that the primary toxicity to guinea-pigs of the antiserum from the rabbit *v.* sheep corpuscles is not parallel with the content in hæmolytic immune body for sheep corpuscles, do not exclude the existence of parallelism between primary toxicity of the antiserum and content in heterophile immune body. The amount of heterophile immune body contained in such sera should be determined by the intensity of their reaction with heterophile lipoids. Of course, the real measure of the reaction caused by heterophile antibody is the difference between the reactions with lipoids of heterophile and non-heterophile tissues (see Table III); the latter being caused by the Wassermann body contained in the antisera (see Taniguchi^{31, 32}).

It must be noted further that the antisera may contain certain normal hæmolytic immune bodies for sheep corpuscles which differ in their properties both from heterophile and isophile immune hæmolysin. Thus it has been shown by Ehrlich and Sachs (1902¹⁰), Bordet and Streng (1909⁴), Yoshinare (1921³⁶), and others, that the hæmolysin for guinea-pig corpuscles contained in normal ox serum is distinct from immune anti-guinea-pig hæmolysin in its susceptibility to heat, and its low affinity for the specific receptors. Similar normal anti-sheep hæmolysin has been observed by the author in guinea-pigs' sera. Thus any or all of such varieties of sheep hæmolysin might be contained in the sera of normal or immunised rabbits; but, since heated sera have been employed, such thermolabile antibodies probably have not been a disturbing factor.

ILLUSTRATIVE EXPERIMENTS.

Examination of the relationship between the content in heterophile immune body and the primary toxicity of hæmolytic immune sera for sheep corpuscles of various origins.

In this and the following experiments, the antisera which were used had been kept frozen in the refrigerator subsequent to heating for thirty minutes at 56° C.

(a) M.H.D. of immune sera was tested for 0·5 c.c. of 3 per cent. sheep corpuscle suspension along with 0·05 c.c. of guinea-pig's complement (the latter always being tested to show that it produced no hæmolysis by itself); (b) for estimating the complement-fixing power; as antigen, 0·5 c.c. of a 10 per cent. emulsion in saline of the crude alcoholic extract (1 part of wet tissue extracted with 9 parts of 96 per cent. alcohol), of medium turbidity, was employed. The mixture of antigen and 0·02 c.c. of heated antiserum was incubated along with ascending amounts of complement for 1·5 hours at 37° C., the tubes being shaken every quarter of an hour. Then 0·5 c.c. of 3 per cent. ox corpuscle suspension sensitised with excess of corresponding immune body (at least 5 M.H.D.) was added to each tube. The results were recorded after further incubation at 37° C. for one-and-a-quarter hours, and standing overnight at room-temperature; (c) in examining for primary toxicity the serum was injected into the jugular vein of guinea-pigs: the procedure being carried out in the course of five minutes.

The sera from rabbits 56, 58, 78, and 79 were drawn off one to three weeks after one injection of guinea-pig's kidney (pure heterophile type of antibody), from rabbits 60, 82, and 83 ten days after the third injection of sheep corpuscles, and from rabbit 65 after one injection (mixed type), and from guinea-pigs 2 and 3 ten days after one injection of sheep corpuscles (pure isophile type).

The results of the estimation of antibody in these sera are given in Table III.

TABLE III.

Serum.	M.H. D. of Immune Body for Sheep Corpuscles in c.c.	Doses of Complement fixed with Alcoholic Heart Extract of			
		Guinea-pig.	Ox (due to Wassermann Body).	Difference (due to Heterophile Antibody).	
Hetero- phile {	Rabbit 78 . . .	0·001	14	0	14
	„ 79 . . .	0 002	12	4	8
	„ 56 . . .	0·002	6	0	6
	„ 58 . . .	0·01	2	0	2
Mixed {	„ 60 . . .	0·0003	26	2	24
	„ 83 . . .	0·001	16	0	16
	„ 82 . . .	0·001	12	0	12
	„ 65 . . .	0·004	6	2	4
Iso- phile {	Guinea-pig 2 . .	0·003	0	0	0
	„ 3 . . .	0·002	0	0	0

For the estimation of the M.H.D. of hæmolytic immune body, the amounts of serum in the successive tubes were in the following ratio—1, 1·5, 2, 3, 4, 5, etc.

In the complement-fixation test, the difference in the amount of complement contained in the successive tubes is 2 M.H.D., and the dose of complement contained in the tube immediately preceding that which gives complete hæmolysis is taken as the amount of complement fixed. When the first tube, which contains two doses of complement, gave complete hæmolysis, then the result was recorded as no complement-fixation. The inhibitory action of antigen and serum each by itself is neglected when two doses of complement cause complete hæmolysis of the test corpuscle suspension.

The results of the examination for primary toxicity of the antisera for guinea-pigs are given in Table IV.

TABLE IV.

Serum from		Weight of Animal.	Amount of Serum injected in c.c.	Result.	Limits between which lie the Lethal Doses per 100 grms. in c.c.
Pure Heterophile	Rabbit 78 . {	200	0.5	Slight effect . .	0.25-0.4
		230	1.0	+ 10 minutes . .	
	,, 79 . {	220	0.5	Marked effect . .	0.23-0.5
		200	1.0	+ 5 minutes . .	
	,, 56 . {	180	0.5	Slight effect . .	0.5-1.1
		200	1.0	Moderate effect . .	
	,, 58 . {	180	2.0	+ 10 minutes . .	
		150	2.0	Slight effect . .	1.4 +
	Mixed {	210	0.5	+ Immediately . .	0.07-0.13
		180	0.25	+ 7 minutes . .	
		180	0.125	Slight effect . .	
	,, 83 . {	200	0.5	Very marked effect	0.25-0.5
		210	1.0	+ 5 minutes . .	
	,, 82 . {	200	0.5	+ 10 minutes . .	0.14-0.25
		180	0.25	Slight effect . .	
Iso-phile {	Guinea-pig 2	200	0.5	Slight effect . .	0.55-1.1
		180	1.0	Moderate effect . .	
		180	2.0	+ 10 minutes . .	
	„ 3	150	2.0	Ø	...
		180	2.0	Ø	

In this table + signifies death of the animal, and Ø no symptoms.

The clinical symptoms and *post-mortem* appearances of the intoxicated animals closely resemble those in anaphylactic shock, as has been agreed by numerous investigators. Milder symptoms, produced by a sublethal dose of antiserum, consist of uneasiness, staring of coat, discharge of faeces and urine, etc. The signs of irritation of skin and mucous membranes (scratching of the nose or body, or coughing and sneezing, etc.) have not been observed so markedly. Dyspnoea or merely increase in the rapidity of respiration is noticeable. The animal may remain in this condition for a time and then gradually recover; increase of body temperature and reduction of serum complement have been observed in such cases. Animals which have received a fatal dose of antiserum may behave as follows: (a) Death may occur during the injection or immediately after without any noticeable symptoms. (b) Typical symptoms appear when death does not occur until after five to ten minutes; the animal falls to one side and exhibits severe dyspnoea, expiration being apparently almost impossible, but evidences of motor irritation (excitement) or general convulsions were not noticed. The respiration weakens and the last movements consist in opening of the mouth and inspiratory widening of the nostrils. In very severe cases the body temperature falls instead of rising as in the milder form.

Post-mortem Examination.—If in such cases the chest is opened after all respiratory movements have ceased, the heart is found to continue beating uniformly and strongly for some time (thirty to sixty minutes), although there is seen to be heart-block. In most cases the heart stops in diastole with dilatation of the right ventricle. Clotting of the blood is usually delayed and

the content of complement in the serum is decreased (viz., M.H.D. of complement for 0.5 c.c. of 3 per cent. ox corpuscles sensitised with 5 M.H.D. of corresponding immune body, before injection = 0.0075 c.c., after injection = 0.015 c.c.). As regards the state of the *lungs*, where death has been immediate, oedema and congestion are the chief features. Loewit (1913²²) and others have stated that the essential difference between the pathological changes in guinea-pigs in which death is due to anaphylactic shock and in those killed by toxic antisera is the absence in the latter of the bronchial spasm with pulmonary emphysema, which is so characteristic of anaphylaxis. But in those animals which have lived some minutes and have developed signs of severe dyspnoea before death, due to the administration of antiserum which possesses primary toxicity, distinct emphysema is noticeable. No marked changes have been noticed in the kidneys and liver except congestion.

The results of the experiments showed that the content of the antiserum in heterophile immune body, as indicated by the intensity of complement fixation with heterophile lipoids, is closely parallel with its primary toxicity for guinea-pigs; although it is perhaps not absolutely so (compare antiserum from rabbits 82 and 83 in Tables III and IV).

As is seen in Table V, the toxic molecules of heterophile antisera can be removed by contact with heterophile antigens (guinea-pig or sheep corpuscles), but not with non-heterophile tissues (ox kidney and corpuscles).

The specific toxicity of the antisera containing heterophile antibody can be demonstrated only for animals of guinea-pig type, which contain heterophile antigen in their tissues (*i.e.*, guinea-pig, dog, fowl, etc.), but not for animals of rabbit type (rabbit, rat, pigeon, etc.), tissues of which do not contain the suitable antigen (Doerr and Pick and others). Animals of guinea-pig type, which are known to be incapable of generating heterophile antibody when injected with heterophile antigen, are also incapable of producing specific toxic molecules, *e.g.*, the antiserum from guinea-pig *v.* sheep corpuscles (see Table IV). Accordingly, the specific toxicity of heterophile antisera for particular animal species must be attributed to the action of heterophile antibody along with suitable antigen contained in the tissues of the host, as held by Sachs and Nathan. Some earlier investigators (Forssman and Hintze) were led to the opposite conclusion, while Doerr and Pick were unable to arrive at a decision, although the results of both sets of observers were similar to those detailed above. This is to be explained partly by their having used the immune serum of the rabbit *v.* sheep corpuscles, which, as has been seen, contains mixed antibody, the isophile moiety of which is, of course, devoid of toxic action. A further cause of their difficulty was that they lacked knowledge of any means whereby to demonstrate specific reaction between the heterophile antiserum with constituents of the tissues of the recipient; thus an analogy with the reaction of the antigen and antibody in protein anaphylaxis (complement fixation or precipitation) seemed to be awaiting.

Any slight quantitative discrepancy in the two properties, as shown by comparing antisera obtained from different animals, might be explained by the presence in certain antisera of other molecules which are toxic to the test animal in a minor degree. Thus it is well known that normal sera, in general, are more or less toxic when injected into different species, or even into other individuals of the same species. Again, the development of toxic action by certain sera is quite distinct from heterophile toxicity; *e.g.*, normal ox serum (unheated) is strongly toxic for rabbits as well as for guinea-pigs (see Doerr and Pick, also personal experiments). Further, there is evidence in support of the view that some specimens of heterophile antiserum were still toxic, to a certain degree, for guinea-pigs after practically complete removal of their heterophile immune body (Table V).

ILLUSTRATIVE EXPERIMENTS.

Primary toxicity for guinea-pigs of heterophile antisera after treatment with heterophile tissues.

Method.—A portion of guinea-pig's kidney is minced and then ground well in a mortar; about 10 volumes of normal saline are then added and the mixture is filtered through gauze to remove coarse particles; thereafter the suspension is repeatedly washed with saline in the centrifuge till the supernatant fluid becomes perfectly clear: equal volumes of the sediment and the antisera are mixed and digested at 0° C. for an hour, being shaken several times. As control, to test the non-specific adsorption of the immune body by tissues, a parallel experiment is carried out with a suspension of non-heterophile tissue, such as ox kidney. To treat the antisera with blood corpuscles, the washed sediment of sheep or ox corpuscles is used respectively.

Antiserum from rabbit 78 was drawn off fourteen days after one injection of guinea-pig kidney; that from rabbit 60 was obtained ten days after the third injection of sheep corpuscles.

For testing toxicity, the antisera were injected into the jugular vein of guinea-pigs of 180 to 250 grms. in weight.

The results of experiments are shown in Table V.

TABLE V.

Serum from Rabbit.	M.H.D. in c.c. of Immune Body for 0.5 c.c. of 3 per cent. Sheep Corpuscles along with 0.05 c.c. of Guinea-pig's Complement.	Amount of Complement fixed by 0.02 c.c. of Serum with Alcoholic-Heart Extract from		Lethal Dose per 100 grms. of Guinea-pig in c.c.
		Guinea-pig.	Ox.	
78 not treated . . .	0.001	14	0	$\begin{cases} < 0.4 \\ > 0.25 \end{cases}$ (1.1 = no symptoms) < 0.44
78 treated with sheep corpuscles	(0.1 = no lysis)	0	0	
78 treated with ox corpuscles	0.001	12	0	
60 not treated . . .	0.0003	25-30	2	$\begin{cases} < 0.13 \\ > 0.07 \end{cases}$ (0.99 = marked symptoms) < 0.11
60 treated with guinea-pig's kidney	0.0008	2	2	
60 treated with ox kidney	0.0004	25-30	2	

THE CHEMICAL NATURE OF THE TISSUE CONSTITUENTS (RECEPTORS)
WHICH REACT WITH HETEROPHILE ANTIBODY.

It has been suggested that the toxicity of heterophile antisera is due to interaction between the heterophile antibody with a suitable antigen contained in the host, *i.e.*, is of anaphylactic character. The specific reactions of heterophile antibody with lipoids of heterophile antigens (complement fixation and precipitation) might throw light on the constituents of the animal body which react *in vivo*. But it should be noted here, that in heterophile antigen-antibody reactions, the power to react specifically with the antibody *in vitro* and the capacity for developing antibody *in vivo* are to a marked extent independent properties; thus lipoids of heterophile antigens are practically devoid of power to generate heterophile antibody. The antigenic character would seem to depend on some lipoid-protein complex, whereas the specific receptors reside in the lipoids (Taniguchi³³). As has been shown by earlier investigators, fresh tissue which can act as heterophile antigen, is capable of fixing heterophile antibody, as well as the toxic molecules, *in vitro*. Experiments also show that the combination of fresh tissue of heterophile type with heterophile antibody leads to specific fixation of complement (Table VI). But whether the lipoid itself can act as receptor for heterophile antibody *in vivo*, or whether it acts when in combination with proteins (which is supposed to be the natural condition of tissue lipoids), is not clear. However, it seems true, that proteins free from specific lipoids are incapable of acting as the receptors in this case, since the protein residue of heterophile antigens, remaining after extraction of the lipoids, does not possess the specific affinity for heterophile immune body, and is also incapable of generating heterophile antibody when injected into suitable animals. Denaturation of the proteins might be adduced in explanation of such loss of properties; but this does not appear to be an adequate reason (see Taniguchi³³). Thus heterophile anaphylaxis would be peculiar in this respect that lipoids played an essential part in the reaction leading to toxic manifestations. It would thus contrast with anaphylaxis in the sense originally described, where the specific receptors are supposed to be of protein nature. The term *heterophile* or *lipoidophile anaphylaxis* has been employed here to designate the former reaction.

ILLUSTRATIVE EXPERIMENT.

Complement fixation due to heterophile antibody along with watery cell suspensions of heterophile tissue.

Antigen.—A 2 per cent. suspension in saline of washed sediment of fresh tissue, prepared as described (p. 86); 0.5 c.c. was used in each tube. Guinea-pig's and horse's kidney represent tissue of heterophile type; ox kidney serves as control.

Antiserum.—0.05 c.c. of heated serum from rabbit 56 (injected with guinea-

pig's kidney : M.H.D. for 0.5 c.c. of 3 per cent. sheep corpuscle suspension = 0.002 c.c.) was used in each tube.

Procedure for the complement fixation test is the same as described on p. 83. The results are shown in Table VI.

TABLE VI.

Antigen : Cell Suspension of	Lysis of Test Corpuscles with the following Doses of Complement.				
	Antiserum + Tissue Emulsion.				Emulsion Control.
	3.	6.	12.	15.	3.
Guinea-pig's kidney .	0	0	m	c	c
Horse kidney . .	0	0	vm	c	c
Ox kidney . .	tr	c	c	c	c

Controls.—Serum alone with 3 doses of complement = ac.
M.H.D. of complement = 0.0075 c.c.

HETEROPHILE ANAPHYLAXIS WITH NORMAL SERA.

According to Friedemann (1917¹⁸) and others, normal sera of man, rabbit, and pig contain varying amounts of hæmolytic immune body for sheep corpuscles, which possesses the character of heterophile immune body, since it is specifically removed by contact with heterophile tissues. Personal experiments also corroborated this fact and afforded further support of Friedemann's view that normal anti-sheep hæmolysin from man and rabbit exhibit specific reactions with lipoids of heterophile tissues (see Taniguchi³¹). Therefore, when normal sera from such individuals, containing a considerable amount of heterophile immune body, were administered parenterally into suitable animals (of guinea-pig type), the development of heterophile anaphylaxis was to be expected. This assumption is supported by the experiment of Doerr and Pick (1913^{6, 7}), who found that the toxicity of normal rabbits' sera was removed by treating with heterophile tissues. As illustrated in Table VII, the amount of sheep hæmolysin contained in heated human sera varies

TABLE VII.

Content of Sheep Hæmolysin in Human and Normal Rabbit Sera.

Sera heated for thirty minutes at 56° C. were tested.

M.H.D. in c.c. for 0.5 c.c. of 3 per cent. sheep corpuscles with 0.05 c.c. of guinea-pig's complement.

Sera.	0.1+.	0.1.	0.05.	0.03.	0.01.	0.01-.	Total.
Human . . .	109	41	29	24	6	2	211
Rabbit . . .	20	12	5	2	1	0	40

within wide limits, depending upon the individual. Out of 211 different specimens, 109 contained a minimal amount, but some were powerfully hæmolytic. Similar individual variations in the content of sheep hæmolysin in normal rabbits' sera have also been observed.

As has been mentioned already, animals of guinea-pig type are incapable of generating heterophile antibody. It has also been stated by Friedemann that normal sera of such animals never contain heterophile anti-sheep hæmolysin; thus normal sera of the guinea-pig, horse, cat, and dog sometimes contain a small amount of hæmolytic immune body for sheep corpuscles, but it is not of heterophile type, because it does not possess specific affinity for heterophile tissues. Personal experiments with the normal immune body of guinea-pig sera corroborated this fact. Then the question arises as to whether or not normal sera of man and rabbit may contain natural sheep hæmolysin of another type in addition to that of heterophile type. Natural sheep hæmolysin contained in the heated sera of these animals has been almost entirely removed by contact with heterophile tissues (Table VIII). Thus the hæmolytic power of such heated normal sera for sheep corpuscles indicates the relative content in heterophile antibody, as they do not contain appreciable amounts of sheep hæmolysin of non-heterophile type.

TABLE VIII.

Absorption of Sheep Hæmolysin of Human Sera with Heterophile Tissues.

Sera were heated for thirty minutes at 56° C.

Procedure is the same as on p. 86.

M.H.D. in c.c. of sera for 0·5 c.c. of 3 per cent. sheep corpuscles with 0·05 c.c. of guinea-pig's complement.

Serum.	Not Treated.	Treated with Emulsion of Kidney Tissue of	
		Guinea-pig.	Ox.
1	0·05	(0·1 = no lysis)	0·05
2	0·003	(0·1 = no lysis)	0·005

Similar results have been obtained with rabbits' sera.

Nothing is known as to the cause of the varying content of heterophile antibody in human sera. Suzuki (1920³⁰) found that natural anti-pig hæmolysin is not usually present in the sera of young albino rats, but appears frequently in older animals, being more abundant in females than in males. He also observed that this hæmolysin is specially increased in pregnancy and decreases after parturition. In certain diseases also, such as infections of the lung, the hæmolysin is increased. According to Obata (1914²⁴), natural anti-sheep hæmolysin is more abundant in women than in men; but it is equally distributed among non-pregnant and pregnant women. In the

case of rabbits' serum the author³² has demonstrated the parallel development of heterophile antibody and Wassermann body when heterophile antigens are injected. Proceeding on this analogy, the content in heterophile antibody (normal sheep hæmolysin) of human sera has been compared with the amount of complement fixed in the Wassermann reaction.

One hundred and thirty-four different sera (heated for thirty minutes at 56° C.), comprising 53 positives and 81 negatives in the Wassermann test, have been examined; but there was no constant relationship between the two properties. Of three sera from quiescent cases of paroxysmal hæmoglobinuria, two, which showed a positive Donath-Landsteiner reaction, contained a considerable amount of heterophile antibody (M.H.D. for 0.5 c.c. of 3 per cent. sheep corpuscles with 0.05 c.c. of guinea-pig's complement = 0.03 and 0.01 c.c.); whereas, one serum which did not contain autolysin, did not have an appreciable action on sheep corpuscles in the presence of complement. But, of course, it is difficult to draw conclusions from such a small series of cases.

HETEROPHILE ANAPHYLAXIS IN THE HUMAN SUBJECT.

The conditions for heterophile anaphylaxis are not provided when sera containing heterophile antibody are injected into the human subject, as human tissues do not contain heterophile antigen. But it has been suggested that horse serum may contain heterophile antigen, in view of the general observation that the serum of animals of guinea-pig type also contains heterophile antigen, although the blood corpuscles of such animals lack this property. This has been confirmed by the author; thus alcoholic extracts of the sera of the horse, guinea-pig, and cat exhibited the specific reaction (complement fixation) with heterophile antisera, whereas those of the rabbit and ox did not. It is to be noted that the sera of animals of guinea-pig type contain much less heterophile antigen than kidney or heart and even less than liver, but nevertheless the amount is by no means negligible. Hence, when horse serum is injected into the human body, it may be expected that interaction will occur between the heterophile antigen so introduced and the heterophile antibody which is frequently present in notable amount and occasionally is abundant in human sera.

Amako succeeded in causing acute shock by injecting sheep or goat corpuscles (heterophile corpuscles) into rabbits which had been previously injected with heterophile antigen from different animal species (organs of guinea-pig, dog, tortoise, and fowl) and had developed a large amount of heterophile antibody in their sera; whereas, the injection of blood corpuscles from the ox, horse, dog, guinea-pig, and tortoise was without effect. The injection of non-heterophile tissues (organs of ox, pig, frog, etc.) did not render rabbits anaphylactic for heterophile blood corpuscles. He also observed that the simultaneous

injection of heterophile antisera with sheep or goat corpuscles into normal rabbits led to acute shock. Therefore, he believed that the development of such intoxication is anaphylactic and supposed that the antibody which is contained in the immunised rabbits' sera, and which renders the host anaphylactic for heterophile corpuscles (*e.g.*, of the sheep), is identical with that which is primarily toxic to guinea-pigs. Thus it is suggested that the injection of heterophile antigen into animals which contain heterophile antibody, leads to anaphylactic symptoms in virtue of the presence of this antibody. By analogy, therefore, it appears to the author that the injection of horse serum into the human subject might be expected to lead to symptoms of heterophile anaphylaxis, the degree of which would depend upon the amount of heterophile immune body present in the individual. As has been mentioned already, the amount of heterophile immune body contained in the serum of different human beings varies within very wide limits; hence, the injection of horse serum might be responsible for serious intoxication in particular cases. This is the reverse mechanism of that commonly supposed to explain the toxicity of horse serum, either normal or immune, for the human subject. Of course, in Amako's experiments, the question of harmful effects due to agglutination of the red corpuscles which were injected, or possibly to toxic substances arising in the course of hæmolysis, must be considered; although no toxic products can be demonstrated when blood is lysed by such procedures as the addition of water or freezing and thawing (see Bayliss, 1920²). Whether or not, heterophile anaphylaxis can develop as the result of injecting heterophile antigens into such human subjects as contain heterophile antibody, requires further investigation. This is an important problem which should not escape attention. In addition to horse serum, heterophile antigens are present in various bacteria which may be injected for prophylactic or therapeutic purposes; thus Rothacker (1913²⁶) found that paratyphoid and Gaertner bacilli contained heterophile antigen.

CONCLUSIONS.

1. The anaphylactic symptoms caused by parenteral administration of heterophile antisera into animals which contain heterophile antigen in their tissues, should be attributed mainly to the interaction between heterophile antigen and the antibody so introduced, although the antisera may also contain other molecules which are toxic to the animal in a minor degree.

2. The specific receptors for heterophile antibody reside in the lipid constituents (lecithin fraction) of heterophile tissues. Watery suspensions of untreated heterophile tissues also exhibit specific reactions with heterophile antibody, but proteins from such tissues which have been deprived of their lipid constituents, lack the property.

Thus it is suggested that the molecules which react specifically with heterophile antibody *in vivo* are lipoids or lipoid-protein compounds, but not proteins free from lipoids. Hence, the mechanism of heterophile anaphylaxis is to be regarded as distinct from that of anaphylaxis as originally described, where the specific receptors for the corresponding antibody are supposed to be proteins. The development of anaphylactic shock by the reaction of specific lipoids and corresponding antibody would thus be established for the first time in the case of heterophile anaphylaxis.

3. Normal sera of certain animals (man, rabbit, etc.) contain a varying amount of heterophile antibody; thus conditions for heterophile anaphylaxis are provided when such sera are injected into suitable animals (*e.g.*, guinea-pig, etc.).

4. The question arises as to whether heterophile anaphylactic reactions may also develop as the result of injecting heterophile antigens (horse serum, certain kinds of bacteria, etc.) into the human subject where naturally a variable amount of heterophile antibody is present.

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