NEUTRALIZATION OF TUMOR VIRUSES BY THE BLOOD OF NORMAL FOWLS OF DIFFERENT AGES*

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A previous publication[13] described the presence of natural antibodies against many bacteria, viruses, and foreign cells in the blood of normal adult chickens, and their absence in that of the chick. The present report deals with the humoral aspects of the natural resistance of the adult fowl to tumor viruses on the basis of the existence of antibody-like substances against such viruses. Facts concerning the development in aging animals of a state of immunity against many infections have been reviewed by Baumgartner.[8]

The knowledge that wide individual variations of susceptibility to tumor viruses exist among normal chickens dates from the discovery of the tumors themselves.[24, 25, 29, 30, 31] Such variations were recognized as ranging from an extreme susceptibility to a complete resistance, and included those cases in which rapid growth was followed by regression and resistance to reinoculation. The amount of inoculum, the age and strain of the host, and the types of tumor were found to be important determinant factors. Moreover, the mode of growth and formation of metastases seemed to be the exclusive result of cell activity. The analogy with what was known of transplantable mammalian tumors was complete and it was only natural that the host reactions to the transplanted malignant cells, rather than to the virus, were chiefly emphasized.[32]

The first worker to approach the humoral aspects of the problem was Carrel.[10] His work established the existence in the blood of normal chickens of some suppressing factor for the Rous virus. This was confirmed by some workers but not by others, and, since the methods used by them varied considerably, they will be analyzed together with the results obtained. Following the finding[33] that the Rous virus rapidly deteriorated when suspended in saline solution at 38° C., Carrel determined that the time necessary for a tumor extract to become inactive at 38° C. was 15 hours. He then studied the retarding influence of sera from chickens and other birds on this spontaneous inactivation, finding that tumor extract, when mixed with sera from young chickens, induced larger tumors than did similar mixtures employing serum of older ones. Andrewes[4] studied the effect of sera from normal chickens on the Rous virus and compared their activity with that of either immune sera or other normal sera. In only a few cases did he estimate the suppressing activity of a given serum by comparing the growth of tumors derived

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from serum-virus mixtures with that of tumors arising after inoculation of virus suspended in saline solution or rabbit serum. In only 2 of 38 fowls could antibodies against the virus be demonstrated. However, in other papers[5, 6] he states that many uninoculated fowls as they grow older develop "normal" antibodies against the Rous virus. In still another publication[7] he reports finding antibodies in a few recently hatched chicks; in this case the antibody was transmitted by resistant mothers through the egg-yolk and persisted in the chick only a few weeks. Sittenfield, Johnson, and Jobling[34] found normal chicken serum "protective." Ledingham and Gye[17] and Amies[1, 2] inoculated groups of chickens with the Rous and Fuginami viruses and obtained samples of serum before and at various intervals after the inoculation. Tests of both the agglutinating and the suppressing powers of the sera were carried out with virus suspensions purified by high-speed centrifugation. The results showed that both attributes were frequently present in the serum of adult chickens, but not in the serum of young individuals. Fischer[14] cultivated Rous sarcoma cells in normal chicken plasma, and injected cell-free material from these cultures into chickens. From the number of successful takes he concluded that 40 per cent of normal chickens have a "virus-antagonistic principle."

Ludford[19] approached the problem in order to explain the unexpected inactivity occasionally shown by the fluid of the culture in which the Fuginami virus was grown. He found in some cases that the plasma exhibited a suppressing effect difficult to ascribe to substances other than the naturally present antibodies. Des Ligneris[12] investigated the effect of normal fowl sera on the Rous virus, with adequate saline controls and prolonged incubation. He concluded that such sera were devoid of action on the virus. Rous, McMaster, and Hudack[27] found that the majority of normal adult Plymouth Rock fowls possess neutralizing substances in their blood, whereas they are much less frequent in pullets of the same stock, and in pooled serum of newly hatched chicks they are not demonstrable. These authors did not publish the details of the method followed.

It is clear from the investigations reviewed above, that the effect of normal fowl sera on tumor viruses was generally made by comparing such sera either with each other or with immune sera. In only a few cases were controls of virus alone employed. In this way a suppressing power of normal sera for the viruses would be overlooked unless it was very pronounced. Still other objections to most of the above methods are the short time of incubation and the method of testing the mixtures by deep injection into the muscles, a location where tumors cannot be accurately measured until the death of the bird. Important initial differences between test and control growths can thus be overlooked.

In those instances in which tests of neutralization in vitro were employed[31] there is unanimity among all workers[10, 12, 21, 34, 36, 37] concerning the presence of potent neutralizing antibodies for the Rous sarcoma in the blood of chickens which manifest an absolute resistance to the tumor (either naturally or after

tumor regression). Agreement is also nearly unanimous concerning the presence of such antibodies in the blood of tumor-bearing chickens. Des Ligneris[12] found them constantly from 8 to 10 days after inoculation, and in greater amount in chickens bearing slow-growing tumors. Gye and Purdy[15] found them only occasionally, but believe that in most cases their level is too low to be detected by the usual methods. Andrewes[4] detected them inconstantly in fowls bearing the Rous tumor, but quite constantly in birds bearing less rapidly growing tumors. Such antibodies were effective against several viruses of the fowl tumor group, and the results obtained served partly as a basis for outlining a serological classification of such viruses.[4, 5] Amies[1, 2] found them, too, together with the agglutinating antibody.

Materials and methods

As has been stated, the fact that the virus suspended in saline rapidly deteriorates on standing at 38° C. has been a serious obstacle to the detection and estimation of viral antibodies in normal sera. The fact, as observed by us, that the action of such antibody takes place at low temperature just as well as at a higher one has afforded a simple means of avoiding this difficulty. Other helpful procedures have been: (a) the use of unfiltered tumor extracts, very rich in virus, after adequate tests had shown that the few cells possibly present were without influence on the results, and (b) the use of the intradermal method which permits of multiple comparative inocula.

Sources of tumor viruses. Rous and Fuginami sarcomata and Mill Hill 2 endothelioma, the two latter kindly supplied by Dr. Gye and Dr. Andrewes, have been used. The tumors were grown for 8 to 10 days in the breasts of 6-month-old Plymouth Rock chickens injected with 0.5 to 1 cc. of fresh tumor pulp. In all cases the tumor was used immediately after killing the chicken. The Rous virus, with which most of the present work has been carried out, was used in the form of saline extracts of the tumor and also after separation from inert tumor components by high-speed centrifugation. Tumor extracts were obtained by grinding the healthy tumor tissue with sand and 19 volumes of saline solution and by centrifuging the resulting pulp for 20 minutes at 3,000 r.p.m. The purified preparations of the viruses, as obtained by high-speed centrifugation, were kindly supplied by Dr. A. Claude. His method[11] can be thus summarized: Fresh tumor pulp was extracted with 15 volumes of distilled water and after a preliminary centrifugation the material was passed through a Berkefeld V candle. The filtrate was then submitted to a centrifugal force of 14,000 times gravity for from one to three hours and the sediment was suspended in Tyrode solution. The coarse particles were eliminated by low-speed centrifugation and the virus was again sedimented at high-speed, these alternating operations being repeated once more. The final material was suspended in a volume of Tyrode equal to that of the original filtrate.

Testing of sera against the tumor viruses. The saline extract of tumor tissue and the centrifuged virus were used in 1:20, 1:200, and 1:2000

dilutions. The serum was generally used undiluted. Equal volumes of the virus suspensions and serum were mixed and left at 2 to 4° C. for 3 hours. After similar mixtures of virus suspensions and saline solution were prepared, 0.5 cc. of each of the 3 serum-virus mixtures were injected into the skin on one side of the breast of adult Plymouth Rock chickens, while the saline mixtures were similarly injected in the other side in corresponding locations. The areas of the tumors resulting from such injections were measured and recorded every week until the death of the animal.

Experimental

Effect on the Rous virus of sera from chickens of various ages

Several experiments were carried out. Since the procedure was essentially the same in all, the results are analyzed together.

Experiments. Sera from (a) 27 chickens 6 to 10 months old; (b) 4 pullets 4 weeks old; (c) 5 chicks 11 days old, and (d) 25 chicks 1 day old were studied. The birds were Plymouth Rocks, Rhode Island Reds, and a cross of both strains. The sera were tested against 1:20, 1:200, and 1:2000 dilutions of saline extracts of tumor by mixing both ingredients at equal volumes, incubating them at 2 to 4° C. for 3 hours, and injecting 0.5 cc. of each of the mixtures into the skin of chickens. In groups (a), (b), and (c) each serum was tested separately, whereas in group (d) sera from 5 chicks were pooled thus making from the 25 chicks, 5 batches of sera which were tested against the virus. Details and results of the experiments are expressed in Table 1.

It is evident that sera from normal adult chickens contain a factor which strongly suppresses the action of the Rous virus and manifests itself by both a lower incidence and smaller size of the tumors. Of the 27 sera tested, 25 exhibited a marked suppressing power. On the contrary, sera from chicks and pullets were devoid of suppressing effect when tested individually, although the fact that two of the batches of pooled sera from newly hatched chicks showed some suppression indicates the occasional activity of the serum of such young individuals.

The rate of growth of locally induced Rous tumors in chickens of various ages

As first observed by Rous and Murphy[31] young fowls are usually more susceptible to the tumor, while in chick embryos it grows with extraordinary speed.[28] Sugiura[35] grafted the tumor to many young chicks with the result that the growth,—which appeared in every case,—developed "relatively faster" than in adult fowls. Experiments were undertaken with a view to learning whether there was

TABLE 1

NEUTRALIZATION OF ROUS VIRUS BY SERA FROM CHICKENS OF VARYING AGES

Average size and incidence of tumors produced by 0.25 cc. of sarcoma extract mixed with 0.25 cc. of chicken serum or saline solution

Age of chickens	No. of chickens	Extract 1:20		Extract 1:200		Extract 1:2000		Time of recording after injection
		Serum	Saline	Serum	Saline	Serum	Saline	
		sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	days
6-7 mo.	27	2.9	13.6	1.6	3.9	0.1	0.8	21
		60%†	100%	44%	90%	11%	60%	
4 weeks	4	7.6	5.0	3.3	3.3	0.5	0.7	15
		100%	100%	100%	100%	100%	100%	
11 days	5	7.7	7.8	4.8	4.4	0.6	0.5	15
		100%	100%	100%	100%	100%	100%	
1 day	5*	8.2	13.0	5.5	2.6	1.2	1.9	21
1 day	5*	7.0	6.5	1.3	1.3	0.6	0.6	21
1 day	5*	2.0	1.8	0.6	0.9	0.0	0.0	21
1 day	5*	0.2	0.9	0.0	0.2	0.0	0.0	21
1 day	5*	0.0	4.0	0.0	0.3	0.0	0.0	21

* Sera pooled.

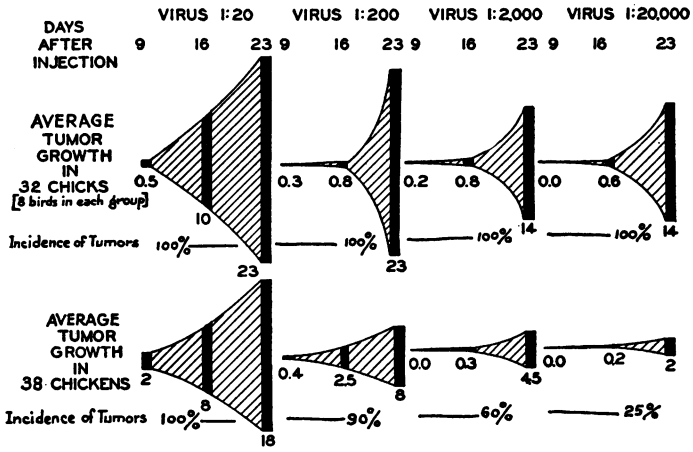
† Percentages refer to incidence of tumors.

a relation between tumor growth and the antiviral factor shown to be present in adult fowls.

Experiment. Dilutions of sarcoma extract at 1:20, 1:200, 1:2000 and 1:20,000 were prepared. Of each dilution 0.5 cc. were injected intradermally into groups of chicks 2 days old and pullets 12 weeks old, each dilution being tested on 8 individual birds. Chickens 8 months old were similarly injected, but here the 4 dilutions were tested on each bird. All birds were of the Plymouth Rock strain. The widely different amounts of virus thus injected allowed comparison of the size of the tumors induced, regardless of whether the virus injected into all birds was the same in amount or proportionate to the body weight of the host. In three different experiments a total of 32 chicks or pullets and 38 chickens was used. The average results obtained are expressed in Fig. 1. The results from chicks and pullets have been considered together.

The figure is self-explanatory. Only when great amounts of virus were injected did larger growths result in chickens than in chicks during the first week or so after inoculation, a result which can perhaps be explained by the larger amount of tissue available for

infection in the adult birds.* Tumors have always grown faster in chicks, and all virus dilutions induced tumors in chicks, whereas several failed to do so in chickens. Individual analysis of the results showed that tumor growth in chicks is subject to individual variations as wide as in adult chickens.



FIGURES UNDER DIAGRAMS INDICATE SIZE OF TUMOR IN SQ.CM.

In summary, the great susceptibility to virus inoculation of the chick as compared with the adult chicken manifests itself by both a constant response to minimal infective dilutions of the tumor virus and by a more rapid growth of the induced tumors.

The effect on the causative virus of sera from adult chickens with differing susceptibility to the Rous tumor

It is known that adult chickens exhibit marked differences in susceptibility to the virus. To explore the possibility that this is referable to differences in the neutralizing power of the blood a group of chickens was inoculated with virus alone and another group with virus which had been incubated with the chicken's own serum.

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* When a large amount of Rous virus, or of several other viruses, is injected intradermally under normal conditions, only a part of it actually induces lesions. This is shown by the enhancement of the lesion when the material is spread through the skin through the influence of added spreading factor.

Experiments. Nineteen normal adult Plymouth Rock fowls and four in which a previously inoculated tumor had regressed were employed. Serum was obtained from each bird, incubated with virus for 3 hours at 2 to 4° C., and 0.5 cc. of the mixture were injected intradermally into one breast, while the opposite breast received the saline-virus suspensions. Virus dilutions of 1:20, 1:200, and 1:2000 were employed. The study was carried out in 3 separate experiments with virus materials which were not of wholly comparable activity. The chickens which developed tumors as the result of these inoculations were observed until death. Those which did not develop tumors or in which initial tumors regressed were later sacrificed. Gross and histological examinations were carried out in all cases and the results are summarized in Table 2.

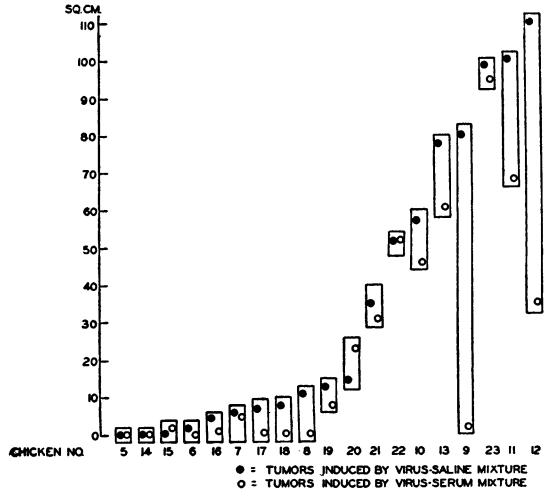

FIG. 2

In Fig. 2 the results of the injections of the virus-saline suspensions in the 19 normal birds have been compared with those of the serum-virus suspensions without regard to the sample of virus employed. The sum of the areas of the three control lesions has been plotted against the same figure for the serum-virus lesions in each of the 19 chickens. The bird number used in Table 2 has been kept in the text figure.

The data presented in Table 2 and in the figure indicate that with the exception of chicken number 9 there is a close direct relation between the size of the tumors induced by the virus-saline and the virus-serum mixtures.* The data show that in practically every instance the serum manifested a suppressing activity, but they do not completely eliminate the possibility that other factors are finally responsible for the clearly defined division of the 19 chickens into resistant (small tumors) and susceptible (large tumors). If, how-

* The results pertaining to metastasis formation as presented in Table 2 will receive comment in a following paper.

TABLE 2

EFFECT OF SERA FROM ADULT CHICKENS ON THE VIRUS OF ROUS SARCOMA AS TESTED ON THE INDIVIDUAL SUPPLYING EACH SERUM

		Area of tumors 21 days after injection of 0.25 cc. of tumor plus									
		0.25 cc. of chicken's own serum			0.25 cc. saline solution						
	Sera No.	Dilutions of tumor extract 1:20	1:200	1:2000	1:20	1:200	1:2000	Time of survival	Macroscopic metastasis	Microscopic metastasis	Observations
		sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.				
1st exp.	1	0.0	0.0	0.0	0.0	0.0	0.0	killed 3 mo. after injection	none	none	
	2	0.0	0.0	0.0	2.5	0.0	0.5	68 days	++++++++++* lung	++++++++++ lung	
	3	0.0	0.0	0.0	5.0	3.5	0.5	killed 12 mo. after injection	none	none	Tumors regressed
	4	0.0	0.0	0.0	9.5	2.0	0.0	30 days	++ lung	++ lung	
2nd exp.	5	0.0	0.0	0.0	0.0	0.0	0.0	killed 3 mo. after injection	none	none	
	6	0.0	0.0	0.0	1.5	0.0	0.0	killed 1 mo. after injection	none	none	Tumor regressed
	7	5.0	0.0	0.0	3.5	2.0	0.0	33 days	none	doubtful	
	8	0.0	0.0	0.0	6.0	3.0	2.5	30 days	+ lung	++ lung	
	9	1.5	0.0	0.0	30.0	25.0	25.0	27 days	none	+++ lung	
	10	25.0	16.0	5.0	31.5	21.0	5.0	25 days	none	none	
	11	35.0	20.0	12.0	51.0	30.0	20.0	23 days	none	++ lung	Lung metastasis of a diffuse infiltrating type
	12	16.0	13.0	6.5	53.0	38.5	27.0	21 days	none	++ lung	Lung metastasis of a diffuse infiltrating type
	13	37.5	16.0	8.0	37.5	27.0	14.0	25 days	none	none	
3rd exp.	14	0.0	0.0	0.0	0.0	0.0	0.0	killed 3 mo. after injection	none	none	
	15	3.0	0.0	0.0	0.0	0.0	0.0	40 days	++ liver	none	
	16	0.6	0.0	0.0	2.0	1.5	0.5	killed 3 mo. after injection	++ lung	none	Tumors regressed
	17	0.5	0.0	0.0	7.5	0.0	0.0	35 days	+++ lung	+++ lung	
	18	0.0	0.0	0.0	6.5	1.5	0.0	27 days	none	doubtful	
	19	5.5	1.5	1.0	10.5	2.0	0.0	30 days	++++ lung	++++ lung	
	20	12.5	7.5	3.5	10.0	5.0	0.1	28 days	none	doubtful	
	21	17.0	10.0	4.0	22.0	13.0	0.0	28 days	none	none	
	22	30.0	19.0	4.0	38.5	11.5	2.5	23 days	none	doubtful	
	23	42.5	29.0	23.5	43.0	32.0	24.0	20 days	+ liver	none	

Sera of the 1st experiment came from chickens in which tumors grew for a time and later regressed.

* 10 + signs indicate an organ studded with metastases; 1 + sign indicates the presence of one or two nodules in the whole organ.

ever, it can be shown that the sera of resistant, or of non-resistant chickens, as the case may be, exhibit marked inhibitory power and weak inhibitory power respectively when tested in a group of birds sufficiently large and so varied in age as to include individuals of all grades of susceptibility, then the factor which determines individual susceptibility may well be considered as residing in the serum of the fowl. That such is the case is shown by the following experiments.

Experiments. Three sera, of which two (a and b) were known to be strongly inhibiting and the other (c) to be only slightly so, were studied. Each serum was mixed as usual with the 3 virus dilutions and, after 3 hours of contact at 2 to 4° C., was tested on one side of several chickens varying in age from 2 weeks to 8 months. The customary control mixtures of saline plus virus were injected into the other side. The route of injection and the results are presented in Table 3.

It is seen that the strongly inhibiting sera exhibited their suppressing effect on the virus without regard to the susceptibility of the host (indicated by the size of the tumors produced by virus alone) and that the action of the slightly effective serum was also consistent throughout the group.

Thus, it would seem from the experiments so far reported that the effectiveness of the injected Rous virus in determining a tumor is, in most instances, conditioned by a factor in the serum which, like an antibody, suppresses the effect of the virus.

Differentiation of the virus-suppressing effect of sera from the ability of such sera to flocculate tissue extracts

Normal sera from most adult chickens contain a factor[13] endowed with a flocculating power for tissue extracts, including extracts of Rous sarcoma.

Since it might be argued that the effect of the serum might be exerted upon some components of the tissue extract which are intimately associated with the virus, and not on the virus itself, the following experiments were carried out employing purified preparations of Rous virus.

Experiment. An extract made of 1 part of tumor mash and 15 parts of water was passed through a Berkefeld filter and submitted to repeated high-speed centrifugation and washing with Tyrode solution. Such operations were carried out by Dr. Claude as described in a foregoing section. After 3 such washings the sediment consisted of a material containing much virus. It was suspended in a volume of Tyrode solution equal to the original volume of the filtrate, and 3 successive ten-fold dilutions in saline were prepared as

TABLE 3

EFFECT OF MIXTURES OF CHICKEN SERUM AND ROUS VIRUS WHEN TESTED ON CHICKENS OF DIFFERENT SUSCEPTIBILITY

Exp. No.	Serum tested	Area of tumors 21 days after injection of 0.25 cc. tumor extract plus: 0.25 cc. chicken serum 1:20	0.25 cc. chicken serum 1:200	0.25 cc. chicken serum 1:2000	0.25 cc. saline solution 1:20	0.25 cc. saline solution 1:200	0.25 cc. saline solution 1:2000	Age of chicken used to test mixtures	Route of injection
		sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.		
I	a	0.0	0.0	0.0	12	3.6	0.0	8 months	Intradermal
		0.0	0.0	0.0	12	3.6	0.0	8 months	Intradermal
		0.0	0.0	0.0	18	4.4	0.0	8 months	Intradermal
		0.3	0.0	0.0	43	6.0	0.0	8 months	Intradermal
II	a		0.0	0.0		2.5	0.0	4 weeks	Intramuscular
		0.0	0.0	0.0	6.0	3.0	0.0	8 months	Intramuscular
		0.1	0.0	0.0	6.0	5.0	0.0	6 weeks	Intradermal
			0.0	0.0		6.3	0.0	2 weeks	Intramuscular
		0.0	0.0	0.0	12	8.8	0.0	4 weeks	Intradermal
III	b	0.0	0.0	0.0	16	0.0	0.0	8 months	Intradermal
		0.0	0.0	0.0	11	2.0	0.0	8 months	Intradermal
		0.0	0.0	0.0	37	3.6	0.0	4 weeks	Intradermal
		6.6	1.5	0.0	37	1.4	0.0	2 weeks	Intradermal
IV	c	7.8	0.8	0.0	9.0	5.4	0.0	8 months	Intradermal
		2.4	0.0	0.0	10	4.0	0.0	8 months	Intradermal
		28	0.0	0.0	28	2.7	0.0	8 months	Intradermal
V	c	0.6	0.0	0.0	5.5	0.0	0.0	8 months	Intramuscular
		12	0.3		8.0	6.2		2 weeks	Intramuscular
		7.5	0.3	0.0	9.8	5.7	3.6	2 weeks	Intradermal
		5.3	0.9	0.1	9.8	4.1	0.3	4 weeks	Intradermal
		7.0	2.3	2.0	10	9.2	1.1	6 weeks	Intradermal
		7.7	0.6		15	8.6		4 weeks	Intramuscular
		7.8	2.1	0.0	22	18	1.4	8 months	Intradermal
			6.3	0.0		11	19	6 weeks	Intradermal

before. Three sera from normal adult chickens were tested against the virus, following the technic employed for tumor extracts. The results may be stated as follows: no tumors at all resulted from the injections of mixtures of sera plus virus, while tumors did result from the injection of virus alone.

These results show that the suppressing action of chicken serum on the virus takes place even if the latter has been freed of much inert matter. However, the results do not completely meet the objection raised, since highly purified virus is known to possess some material which immediately reacts as a normal component of chicken tissues.[1] Accordingly, the following study was carried out.

Experiment. Twenty chicken sera, whose suppressing effects on the virus were previously known, were tested for their content in the factor which flocculates tissue extracts. Extracts of one part of mouse liver or of Rous tumor with 19 parts of saline solution were obtained and centrifuged. From the supernatant fluid progressive serial dilutions from 1:20 to 1:320 were prepared, and to 0.5 cc. of each of these, 0.1 cc. of one of the chicken sera was added. After mixing, the materials were left overnight at from 2 to 4° C., when they were allowed to warm at room temperature. The tubes were then shaken and the degree of flocculation was recorded. Flocculation of the liver extract was always more pronounced than was that of the tumor extract. With each serum a comparison of the flocculating power for tissue extracts with the suppressing power for the Rous virus showed complete disagreement in about 50 per cent of the cases, and in those sera exhibiting both actions a lack of a quantitative relation between the properties was often evident.

The experiments show that the factor which flocculates tissue extracts is not responsible for the neutralization of the virus.

Some characteristics of the suppression of tumor virus by chicken serum

While a full study of the physical and chemical properties of the factor neutralizing the Rous virus is under way in collaboration with Dr. K. G. Stern, it can be said in passing that the factor resides in the slowly migrating globulin fraction where serum antibodies are usually found. The factor exerts its effects on the virus at 37° and 42° as well as at 2 to 4°. A certain period of contact between serum and virus is necessary, the suppression of the latter being weak or absent if the mixtures are injected without incubation, but the suppression is no greater after 20 hours than after 4 hours of contact. Active sera stored for several weeks in the refrigerator retained their neutralizing power, and both fresh and stored sera still exerted some suppression when diluted 50 times, and in some instances even when diluted 1000 times. The suppressing power in the blood of normal

chickens is inferior to that observed in the blood of chickens in which a Rous tumor had regressed. It is not altered in chickens injected with tar or chemical carcinogens even though these substances elicited tumors.

Experiments on the Mill Hill 2 endothelioma and the Fuginami sarcoma

These experiments were devised to discover whether the serum from normal adult chickens is effective in neutralizing chicken tumor viruses other than the Rous virus.

Mill Hill 2. Sera from 9 normal adult chickens were tested against saline extracts of a recently excised Mill Hill 2 endothelioma.[9, 22] The tests were conducted as described in the foregoing experiments in which each mixture of virus and serum was tested on the chicken from which the serum was obtained. Details and results of the tests are given in Table 4.

TABLE 4

EFFECT OF SERA FROM ADULT CHICKENS ON THE VIRUS OF MILL HILL 2 ENDOTHELIOMA AS TESTED ON THE SAME INDIVIDUAL SUPPLYING THE SERUM

Areas of tumors 20 days after injection of 0.25 cc. tumor extract plus

	0.25 cc. chicken's own serum			0.25 cc. saline solution			
	Dilutions tumor extract						
No.	1:20	1:200	1:2000	1:20	1:200	1:2000	Time of death of injected chickens
	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	
1	0.2	0.0	0.0	3.6	0.3	0.0	23 days
2	1.8	0.0	0.0	5.4	0.0	0.0	34 days
3	3.6	1.0	0.3	6.0	2.0	0.0	27 days
4	6.4	0.5	0.0	14.0	0.2	0.0	33 days
5	14.0	4.4	0.0	18.0	9.0	0.0	Tumors regressed
6	19.0	1.5	0.5	21.0	4.2	6.4	27 days
7	28.0	5.0	0.5	27.0	4.0	0.0	20 days

It is seen that in 6 of the 7 instances where tumors developed, the serum exerted a suppressing effect on the virus, although complete suppression of all of the 3 virus dilutions, so often seen in tests on the Rous sarcoma, was not observed. As in the case of the latter virus, the suppressing effect is more pronounced in those chickens which, as judged by the growth of tumors induced by the virus alone, are the more resistant to it.

Fuginami. Sera from 10 normal adult chickens were tested, as above, against saline extracts of freshly excised Fuginami sarcoma. In one of the experiments the sera were tested both undiluted and diluted 1:10, the two

TABLE 5

EFFECT OF SERA FROM ADULT CHICKENS ON THE VIRUS OF FUGINAMI SARCOMA AS TESTED ON THE SAME INDIVIDUAL SUPPLYING THE SERUM

	Serum No.	Tumors resulting from the injection of: Undiluted serum plus tumor extract — Dilutions of tumor extract 1:20	1:200	1:2000	Serum diluted 1:10 plus tumor extract — 1:20	1:200	1:2000	Saline solution plus tumor extract — 1:20	1:200	1:2000	Time of death of injected chicken
		sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	
1st exp.	1	0.0	0.0	0.0				0.0	0.0	0.0	Survived
	2	0.0	0.0	0.0				0.0	0.0	0.0	Survived
	3	0.7	0.0	0.0				9.0	0.0	0.0	32 days
	4	11.0	0.3	0.0				18.0	0.0	0.0	27 days
	5	15.0	3.0	0.3				20.0	1.4	0.0	27 days
2nd exp.	6	0.2	0.1	0.0	1.0	0.2	0.0	0.5	0.3	0.0	Tumors regressed
	7	3.3	0.0	0.0	2.5	0.0	0.0	6.4	0.0	0.0	Tumors regressed
	8	15.0	5.8	2.2	6.0	3.0	0.8	22.0	9.0	0.0	22 days
	9	30.0	2.5	0.0	9.7	9.7	1.2	27.0	4.0	4.0	21 days
	10	8.7	6.6	0.0	4.0	1.6	0.0	32.0	0.0	0.0	27 days

In the 1st experiment the mixtures of serum or saline and tumor extract were injected in a dose of 0.50 cc.; in the 2nd experiment the dose was 0.25 cc.

sets of serum mixtures being injected intradermally in one breast of the chicken. The saline mixtures were injected in the other breast. Details and results of the experiments are expressed in Table 5.

It is seen that in all cases sera from normal chickens showed a suppressing power for the Fuginami virus, and that this suppression was more pronounced in those chickens in which the virus as such induced smaller tumors or produced none at all. However, as in the case of the Mill Hill 2 endothelioma, a complete suppression of all of the 3 virus dilutions was not observed. The results suggest a prozone effect, since sera diluted 1:10 frequently exerted a more pronounced suppression on the virus than did undiluted sera.

The viruses of the Mill Hill 2 endothelioma and the Fuginami sarcoma are suppressed by sera from adult normal chickens, but the effect is less pronounced than in the case of the Rous virus.

Discussion

The study of naturally occurring antibody-like factors which exhibit a suppressing action on filterable viruses has so far received little attention. In our studies a factor behaving to all intents and purposes like a natural antibody for the virus of the Rous sarcoma has been found to be generally present in the blood of adult Plymouth Rock chickens, whereas it was but occasionally found in the blood of chicks. Analogous suppressing factors for the viruses of the Fuginami sarcoma and the Mill Hill 2 endothelioma were also found in the blood of adult fowls.*

It has also been shown that the susceptibility of the chick, as compared to that of the chicken, to the Rous virus manifests itself by a constant response (tumor formation) to very small amounts of inoculum and by the development of larger tumors. A very small percentage of adult chickens are highly susceptible to the virus, and these, like the chicks, show very little or no antibody in the blood, whereas those more resistant or totally resistant show such antibody in amounts directly proportional to their degree of resistance. The latter holds true also for the Fuginami and Mill Hill 2 viruses.

* Speculation on the origin of these factors, and of many other serological and immune factors present in the blood of adult chickens,[13] would lead us to the debated problem of the origin of the natural antibodies in many animal species. Are they serological manifestations of physiological growth and aging or are they the result of subclinical infection? Possibly the first supposition would fit better with what we know about the regularity and, in some cases, the very early appearance of natural antibodies in the chicken, and also with the fact that in many cases the agents acted upon by such antibodies are not concerned with common poultry infections.

The results show that a humoral factor has a great influence in conditioning the infection induced by these tumor viruses, and suggest, but do not prove, that the responsible factor is a viral antibody. One may infer that the injected virus is neutralized by the humoral factor before the former becomes associated with the susceptible cells or when it emerges from the tumor to infect normal surrounding cells.* If the latter supposition is correct it would follow that such a mechanism of tumor growth may be operative in the development of tumors in hosts lacking the neutralizing factor, that is, in chicks and in very susceptible adults. If one accepts, as most workers do, that once the virus becomes associated with the susceptible cell it is invulnerable to surrounding antibodies,[14, 23, 26] speculation on any other effect of the virus-suppressing factor on the primary tumor would be unwarranted.

Two additional pertinent points should receive comment. First, the present findings may well deserve consideration in attempts, such as those of Andrewes,[4, 5] to classify chicken-tumor viruses by serological methods. Second, since plasma from adult fowls is often used in the cultivation of the viruses of tumors and other diseases, a question may be raised as to whether different results might be obtained were chick serum to be used. Ludford's[18, 19] observation that plasma suppressed the culture in vitro of the Fuginami virus may be recalled in this connection.

Summary

Paralleling the growth and aging of the individual there develops in the blood of fowls an antibody-like factor endowed with the property of pronouncedly suppressing the effects of the viruses inducing the Rous and Fuginami sarcomas and the Mill Hill 2 endothelioma.

In the case of the Rous virus, the only one studied in this respect, the factor is rarely present in young chicks, but is to be found almost uniformly in adult chickens. In both groups its presence or absence, or the extent to which it is found, stands, as a rule, in an inverse relationship to the incidence of tumors after inoculation and to the rapidity of growth of the induced tumors. The same has been found to hold true for adult chickens of differing susceptibility to the virus; those developing a slowly growing tumor or no tumors at all have a large amount of the antibody-like factor, while those

* The same mechanism has been suggested in explanation of the satisfactory results obtained in influenza infection treated with antisera.[16, 20]

developing more rapidly growing tumors have lesser amounts of the neutralizing factor or none at all.

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