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## Transplantability and Presence of Virus in Spontaneous Sarcomas and Fibromas of Chickens in Relation to the Age of the Tumor-Bearing Animal\*

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A main interest of avian cancer lies in the fact that in a single species, the chicken, one finds tumors with differing transplantability. On the one hand, tumors such as some sarcomas, endotheliomas, leukotic and lymphoid neoplasms are found that are easily transplantable and undoubtedly induced by viruses; and on the other, tumors, the transplantation of which is either very irregular or very difficult, such as some other lymphoid neoplasms and the mixed embryonal and epithelial tumors are found. A third group comprises tumors which can be transplanted but in which no virus can regularly be demonstrated, such as the sarcomas induced by chemical carcinogens.

This situation may lead the investigator to two radically different points of view: (a) assuming that other agents than viruses can induce cancer even of the same type in the same animal species; (b) assuming that viruses are the cause of all chicken tumors although they cannot be demonstrated in some tumors because of influences still to be determined. The latter supposition could also be applied to all or a part of mammalian neoplasia.

During the last 5 years we have collected about 50 spontaneous chicken tumors and tried to relate their behavior on transplantation and the presence of viruses in them with other characteristics not only of the neoplasms but also of the hosts that had developed them. In the present publication we report results of a study of 14 sarcomas and 7 fibromas. It will be seen that the age of the host may be a factor conditioning the transplantability of the sarcomas.

The incidence of cancer in chickens is very high, perhaps the highest in any species under natural con-

ditions, the disease comprising a great variety of types comparable in every respect to cancer in mammals. Neoplasia of mesenchymal tissues is far more common than epithelial neoplasia and, contrary to the latter, may occur at a very early age (6, 11). A myelocytoma (11), lymphomatosis (6), a fibroma (11), and a sarcoma (1) have been observed in chicks of from 13 days to 5 weeks of age.

Many spontaneous sarcomas can be considered as being indefinitely transplantable and causative viruses have been shown in all of them but unfortunately either no data or only brief statements are available concerning the characteristics of both host and tumor in the cases where transplantation was attempted but failed. Nor are data available in most cases concerning the age of the host in which the transplantable tumors were discovered. We know, however, that sarcomas 1 and 7 of Rous and his associates occurred in chickens 15 and "apparently" 12 months old, the same age as one of Teutschslaender chickens (2). Jungherr's sarcoma and endothelioma (9) and Furth's neoplasms that originated his strains 2, 5, 11, 12, 13, and 15 (7), combined with leukoses or lymphomatosis, were found in chickens under 1 year of age. When the age is not stated no mention is made by the authors that the hosts were either particularly old or young. This holds true for most of the tumors listed by Claude and Murphy (2), the 2 sarcomas of Reis (12) and the 23 of Troisier (14).

Metastases in the original host were present in 12 of 19 cases that we have reviewed. On transplantation all of the 19 tumors induced them. Metastasis ranged from rare, as in the osteochondro-sarcoma of Rous and Lange and the fibrosarcoma MH. 1 of Begg, to frequent as in the Rous sarcoma and so many others (2).

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Olson and Bullis described 25 sarcomas but without attempting transplantation; 21 of them were found in chickens of from 12 to 52 weeks while the rest occurred in chickens of from 60 to 79 weeks. Metastases were present in 14 cases (11).

#### MATERIALS AND METHODS

Our material consisted of 14 sarcomas and 7 fibromas out of about 50 neoplasms found in Plymouth Rocks, Rhode Island Reds, or their hybrids reared in farms around New Haven, Connecticut, most of them coming from the same farm.<sup>1</sup> All the birds were females. In most cases the chickens were brought alive to our laboratory and killed immediately before the tumors were removed and processed. Suspensions of 1 part of cells and 4 of saline solution were prepared from each tumor, while extracts of 1 part of tumor and 19 of saline solution filtered through Berkefeld N candles were obtained from 10 of them.

Cell suspensions were injected in the breast of chickens as follows: A total of 1 cc. into chicks from 7 to 20 days of age, and 2 cc. into pullets from 1 to 2 months of age, half of the inoculum being injected into each breast. When chickens from 4 to 8 months were available 2 of them were additionally inoculated with 4 cc. of the cell suspension prepared by the same method. Filtrates were injected in the same amounts into the breast of from 5 to 10 chicks and pullets. Often 5 additional chicks were injected with 1 cc. into the jugular vein.

Some or all of the birds inoculated belonged to the same breed as the chicken bearing the original neoplasm. Often, however, chickens of other breeds were successfully employed. Cell suspensions from several of the tumors were also injected into the breast of 24 hour old ducks in the amount of 3 cc.

The birds were observed at least once a week. They were killed and autopsied after 50 days if no signs of disease were evident. Accidental losses, sometimes considerable, reduced the number of animals available for analysis to the figures expressed in the tables.

#### RELATION BETWEEN TRANSPLANTABILITY OF THE SARCOMAS AND THE AGE OF THE CHICKENS BEARING THEM

In Table I the tumors have been listed according to the age of the host and, when the age was the same,

<sup>1</sup> It was not possible to determine the incidence of tumors and leukoses in the total chicken population of this farm. The majority of tumors were discovered from December to June inclusive, but it is doubtful whether this is an indication of a seasonal influence since many other factors were at play.

One of the neoplasms, tumor W1, was found and studied by Dr. E. W. Shrigley. It developed in a chick secured from a commercial hatchery.

according to the success met with on transplantation of the tumors. The age of the host was fairly well known except for tumors B and C, in which cases only an approximate age could be obtained. The histological malignancy was appraised by the canons of orthodox pathology from complete absence to the highest degree by signs ranging from 0 to + + +.<sup>2</sup> None of these neoplasms showed the latter extreme of morphological malignancy.

The figures on transplantation indicate the number of chickens that developed tumors in relation to the total number inoculated. These data are given only when they contribute to understand the success or failure of the transplantation.

The most important data on the tumors needed for discussion are summarized in Table I. However, additional information on some of them seems necessary, although the tumors are thoroughly described in the following articles.

The indefinitely transplantable tumors V 1, D 1, and B were soft, fast-growing, infiltrative, and metastasizing, very much resembling the Rous sarcoma, while tumor C, a firm growth, was of a less malignant sort, growing slowly and inducing metastases but rarely.

Judging by the original growth and the growths of the first passage tumors Z and G also seemed to be of a malignant, fast-growing sort. Tumor Z was a rather soft mass filling up the whole breast of the bird and had induced widespread metastases; tumor G was a soft, viscid mass 4 × 4 cm. in the orbit, which had invaded the bone and had formed many metastases in the lungs.

Chickens bearing tumors E and D had large embryonal nephromas and several small sarcoma nodules in the muscles. Portions of the breast growth of tumor E secured by biopsy were indefinitely transplantable and were like the Rous sarcoma. The breast nodules of tumor D were soft, viscid, cystic, and microscopically like the fast-growing indefinitely transplantable sarcomas. Whether these sarcomas can or cannot be considered as metastases of the kidney tumor will be considered in a following publication.

The presumption that tumors Z, D, and G could have been transplanted indefinitely, although actually they were not, is based on the following: tumor Z was not transplanted until at least 10 hours had elapsed after the death of the chicken. Several chicks inoculated with tumor D died accidentally so only 1 chick and 2 full grown chickens were left. Tumor G grew very well in the first transplant but only 1 tumor of this passage was transplanted and several of the inoculated chicks died prematurely. On the other hand we

<sup>2</sup> We are grateful to Dr. H. Bunting for the grading of the malignancy of these neoplasms.

have seen that the 3 tumors showed the characteristics of malignancy also present in 4 of the 5 tumors that were indefinitely transplantable.

Keeping this in mind one could interpret the results from Table I in the sense that transplantation of sarcomas is best achieved when coming from chickens of from 5 to 10 months of age, which are those of group 2. Next are those of group 3, from chickens of about 3 to 18 weeks, and finally those of group 1 from chickens between 12 and 24 months. Chance may

In tumors V 1 and B, cell-free preparations were active from the first passages and in tumor E from the original tumor. In slow-growing tumor C, virus was only occasionally demonstrated while tumor D 1 may occupy an intermediate position. Filtrates from the nontransplantable tumors were consistently ineffective.

2. All of these 5 sarcomas produced metastases in varying degree in the original host and, as will be detailed elsewhere, also in the hosts bearing the transplants. Tumors G and Z also metastasized but prob-

TABLE I: TRANSPLANTABILITY OF SPONTANEOUS SARCOMAS FROM CHICKENS OF VARYING AGE

No.	Age of tumor-bearing chicken	Designation	Data on tumors			Behavior in passages by cell suspensions					
			Type	Histological malignancy	Metastases	1st	2nd	3rd	Indefinite transplantation	Effectiveness of filtrates or desiccates	Indefinite transplantability
Group I											
1	24 mo.	0	Fibromyxosarcoma, skin	++	none	-0/9			-	-	-
2	18 "	V 1	Fibromyxosarcoma, ovary	+	many in peritoneum	+5/8	+	+	+	constant	+
3	14 "	A 1	Myxosarcoma, intestine	+	none	-0/10			-		-
4	12 "	Y 1	Fibrosarcoma, wing	++	"	-0/8			-	nil	-
Group II											
5	6-10 "	C	Fibrosarcoma, breast	+	several in viscera, skin one in lung	+15/15	+	+	+	occasional	+
6	8 "	D 1	Fibromyxosarcoma, breast	+	"	+1/9	+	+	+	constant	+
7	6-10 "	B	"	+	many in peritoneum	+6/6	+	+	+	"	+
8	5-6 "	Z	Fibrosarcoma, breast	++	widespread	+1/7	-0/9		-	"	+‡
9	5 "	E	" and embryonal nephroma	++	*	+4/5	+	+	+	"	+
Group III											
10	18 wk.	Z 1	Fibrosarcoma, orbit	+	none	+3/13†			-	nil	-
11	16-20 "	D	" and embryonal nephroma	+	*	-0/3			-	"	+‡
12	6-8 "	H	Neurogenic sarcoma abdominal plexus	+	none	-0/6			-	"	-
13	5-6 "	G	Fibrosarcoma, orbit	++	many in lung	+5/5	+2/4†		-		+‡
14	23 days	W 1	" thigh	++	none	+6/9	-0/14		-		-

\*The relation between the 2 types of tumors is discussed in the text.

†Tumors regressed.

‡Indefinite transplantability presumed.

have favored this interpretation. If the only chick that responded to grafting of tumor D 1 of group 2 had died prematurely, that tumor would have been considered as nontransplantable. On the other hand, reasons have been given for considering the loss of tumors Z, D, and G from groups 2 and 3 as due to accidental causes.

RELATION BETWEEN TRANSPLANTABILITY AND OTHER CHARACTERISTICS OF THE TUMORS

1. A causative virus was demonstrated only in the 5 sarcomas that proved to be indefinitely transplantable.

ably accidental factors were responsible for failure of indefinite transplantation. Possibly, tumors E and D were themselves metastases of embryonal tumors.

3. The hemorrhagic disease was present in the transplants of all the fast-growing transplantable sarcomas and absent in tumor C. This disease developed in young chickens either after intravenous injections of filtrates or with a generalization of primary tumors. The lesions were in every way comparable to those previously described in other tumors (4, 5).

4. The 4 fast-growing sarcomas, and also tumor G, grew easily in 24 hour old ducks after one or several passages through chickens while slow-growing tumor

C grew in only one of many ducklings injected. We are not concerned here whether adaptation to the foreign species was obtained or not since this belongs to a different study. As will be detailed in another publication, all of the 5 transplantable tumors grew equally well in the breed of chickens in which the tumor originated, such as in chickens of other breeds. Tumors D and Z were inoculated into ducklings with negative results.

5. Indefinite transplantability was constantly associated with histological malignancy since the 5 sarcomas showing that trait were tumors of + or ++ of our grading. However, the reverse was not true, because 7 other sarcomas (excluding tumors Z, D, and G) of a comparable histological type were either not transplantable or were so for only 1 or 2 passages. As for the influence of the size, tumors V 1, C, D 1, and B all measured 10 × 10 cm. or more and were

induced metastases. The latter were likewise absent in all the other fibromas. The histological grading of the degree of malignancy of all the tumors of this group was zero.

#### ADDITIONAL STUDIES ON VERY OLD CHICKENS

We had the opportunity of securing 5 Rhode Island Red chickens aged from 4 to 5½ years. There were 4 hens and 1 rooster, all in excellent condition. When sacrificed, neoplastic lesions were found in all of them as follows:

*Hen No. 1.*—Two fibromyomas each measuring 2 × 2 × 2 cm. attached to the outer wall of the oviduct.

*Hen No. 2.*—A fibroadenoma 6 × 6 × 5 cm. attached to the inner side of the lower abdominal wall and a soft angiomatous growth 1.3 × 0.4 cm. in the upper eye lid.

TABLE II: TRANSPLANTABILITY OF SPONTANEOUS FIBROMAS FROM CHICKENS OF VARYING AGE

No.	Age of tumor-bearing chicken	Data on tumors			Behavior in passages by cell suspensions			
		Designation	Type	Histological malignancy	1st	2nd	3rd	Indefinite trans-plantation
1	30 mo.	X	Fibroma, skin	0	-0/10			-
2	24 "	T	" "	0	-0/9			-
3	18 "	A 2	" "	0	-0/11			-
4	8-9 "	K	Fibromyxoma, breast	0	-0/4			-
5	8-9 "	N 1	Fibroma, costal edge	0	-0/8			-
6	8-9 "	C 1	Fibromyxoma, wing	0	-0/9			-
7	8-9 "	Q 1	Fibromyxoma, orbit	0	+4/12*	+1/16	-0/7	-

NOTE: Effectiveness of filtrates was nil.

\*Two of the tumors regressed.

indefinitely transplantable while the nontransplantable sarcomas were smaller. However, tumor E was also smaller. Tumor W 1 measured only 10 × 4 mm.

6. In 3 cases where adult chickens besides chicks and pullets were inoculated with cell suspensions of the original tumors, growth followed in only 1 chicken injected with cells of tumor VI whereas 5 others injected with cells of either this tumor or of tumor E failed to respond entirely.

#### TRANSPLANTABILITY OF FIBROMAS

The results of transplantation of 7 of these neoplasms are summarized in Table II. Only one was carried through 2 passages. A third passage failed in a rather large number of chicks. The original growth had entirely replaced the right eye and had considerably enlarged through pressure the ocular cavity but had not invaded it. The transplants grew very slowly and like the original tumor were very firm and never

*Hen No. 3.*—A black, firm, ovoid tumor 1.2 × 0.5 cm. loosely attached to the lower pole of the ovary consisting of fibromatous cells together with other cells loaded with pigment.

*Hen No. 4.*—A fibromatous, cystic mass 1 cm. in diameter at the base of the neck.

*Rooster.*—(a) A lymphoblastoma 1.5 cm. in diameter, firm, well capsulated in the wall of the duodenum; (b) nodules of a similar lymphoid tissue distributed throughout the salivary gland; and (c) a horny wart, mostly fibromatous, in the comb.

In summary, the 5 birds showed 9 neoplastic lesions, 8 of them clinically and histologically benign. The ninth lesion, the duodenal lymphocytoma, was clinically benign but many of the cells were in mitosis and histologically the growth showed some malignant traits. There were also areas of degeneration and of old hemorrhage in many tissues of all the birds.

Cell suspensions from 2 of the fibromas and extracts of several tissues from the rooster were injected

into 8 chicks and 2 chickens without tumor development. Scarification of the comb of 4 chickens with an extract of the wart induced no lesions.

It will be noticed that all the fibromatous lesions occurred in full grown or old chickens. However, fibromas in young chickens have been recorded (6).

#### DISCUSSION

A state of maximum malignancy in spontaneous chicken sarcomas manifested itself by (a) indefinite transplantability into homologous hosts of various breeds and by growth in heterologous hosts; (b) by infiltrating growth and metastasis; and (c) by characteristic histological signs. All these features were constantly associated with the presence of free virus as if the quantity or quality of the latter conditioned the behavior of the infected cell. Also the incidence of hemorrhagic lesions, the non-neoplastic manifestations of free sarcoma viruses in young hosts, was always parallel to the effectiveness of cell-free preparations from the tumors. These points will be fully developed in a forthcoming publication.

As for the influence of the age of the host on the transplantability of the sarcomas, it should be pointed out that since our subject material is obviously heterogeneous and consists of only 14 tumors, a final interpretation of the results is subordinated to the outcome of other studies now being carried out with a more homogenous material, intending to prove what is only suggested in the present investigation. With the proviso, the following comments seem pertinent:

From our data it would seem that chicken sarcomas find most frequently their maximum malignancy in hosts from 5 to 10 months of age. This was the degree of malignancy actually found in 4 of 5 sarcomas of group 2 while it could be presumed in the fifth sarcoma. However, at least 3 analogous sarcomas were found by other workers in chickens from 12 to 15 months of age and by ourselves in a chicken 18 months old. This may suggest that the upper limit of transplantability should be raised although it is also probable that some of these cases are exceptions to a rule, a fact little surprising if one considers the relativity of all manifestations of ageing. Also, the numerous neoplasms found in chickens of from 4 to 5½ years old were histologically or clinically benign, whereas the other 7 fibromas of our series occurred in adult or old chickens. Altogether, the results may indicate that the age of the host had exerted a restraining effect on the development of malignant traits by all these tumors. This would be quite in line with the acquisition of resistance against many infections and especially against tumor viruses (3, 5, 10).

None of the 5 sarcomas of group 3 found in chicks of from 23 days to 18 weeks of age could be indefi-

nately transplanted. However, one could presume this transplantability in 2 of them, 1 of which, tumor G, was actually carried through 2 passages. The neurogenic sarcoma, like the fibromas, may have intrinsic characteristics of only relative malignancy (11). In the case of tumors Z1 and W1, ordinary fibrosarcomas, one feels that their cells were given ample opportunities for multiplication yet they could not be carried beyond one passage.

This may suggest that in these 2 tumors, and possibly others of group 3, the cells have not yet attained sufficient malignancy so that they can adapt themselves to other hosts. These results could in a way be compared to those of Green (8) on mammary gland carcinomas of rabbits in which transplantability was not achieved until the tumors had reached a certain stage of development and had become generalized.

It will be noted that in all tumors that were neither actually nor presumably transplantable, from old or young hosts, metastases were absent. Morphologically, these tumors looked just as malignant as the transplantable sarcomas, so that it appears that what can properly be called functional malignancy in chicken sarcomas is to a large extent independent from morphological malignancy.

As for the fibromas our observations show that only 1 of them could be carried through 2 passages. This tumor looked histologically just as benign as the others, and occurred in a chicken 8 or 9 months old. All of our fibromas were found in chickens of that age or much older and none had induced metastases. This may be an indication of a restraining influence from the host or an inherent characteristic of the tumor.<sup>3</sup>

#### SUMMARY AND CONCLUSIONS

Fourteen spontaneous chicken sarcomas have been grouped according to the age of the chicken bearing the neoplasm. The presence of a causative virus, the transplantability of cells and other properties of the tumor have been studied.

Of the 4 sarcomas found in chickens of from 12 to 18 months of age (group 1) only one proved to be indefinitely transplantable and a causative virus could be demonstrated in its filtrates.

Of the 5 sarcomas found in chickens of from 23 days to 18 weeks of age (group 3) none could be trans-

<sup>3</sup> In collaboration with Dr. H. S. N. Greene it has been found that nontransplantable sarcomas, like tumor O and also some embryonal nephromas, can be successfully grafted into the anterior chamber of the eye, from homologous and heterologous hosts. The method could be developed to detect possible malignant potentialities in chicken tumors not disclosed by ordinary transplantation. On the other hand, tumors may undergo variation in the eye of foreign species as exemplified by the case of the Rous sarcoma (13) and it is debatable if analogous changes could take place in the original host.

planted indefinitely although there are reasons to presume that 3 of them could have been so transplanted—one being carried through 2 passages—while 2 others were carried through 1 passage each.

Of the 5 sarcomas found in chickens of from 5 to 10 months of age, 4 proved to be indefinitely transplantable with causative viruses demonstrated in their cell-free preparations, and the same could be presumed in the fifth sarcoma.

Of the 7 fibromas which occurred in chickens of from 8 to 30 months of age, only one, in an 8-9 month chicken, could be carried through 2 passages.

Although indefinite transplantability and subsequent presence of free virus were traits of sarcomas histologically classed as malignant, another 7 sarcomas with the same or a higher degree of histological malignancy failed to show these features while the fibroma carried through 2 passages was histologically nonmalignant.

In general a state of high malignancy in chickens was constantly associated with large, metastasizing sarcomas easily transplantable to chickens of several breeds and also to foreign species, namely, newborn ducks. These tumors were found most frequently in mature but not old chickens. Presumably, they may occur in some young chickens also. In all the indefinitely transplantable tumors, neoplastic viruses, their causative agents, were demonstrated.

#### REFERENCES

1. BALL, R. F., and HUFF, F. B. A Spontaneous Fibrosarcoma in a Thirteen-Day-Old Chick. *The Cornell Veterinarian*, **34**:254-256. 1944.
2. CLAUDE, A., and MURPHY, J. B. Transmissible Tumors of the Fowl. *Physiol. Rev.*, **13**:246-275. 1933.
3. DURAN-REYNALS, F. Neutralization of Tumor Viruses by the Blood of Normal Fowls of Different Ages. *Yale J. Biol. & Med.*, **13**:61-76. 1940.
4. DURAN-REYNALS, F. A Hemorrhagic Disease Occurring in Chicks Inoculated with the Rous and Fuginami Viruses. *Yale J. Biol. & Med.*, **13**:77-98. 1940.
5. DURAN-REYNALS, F. The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses. *Cancer Research*, **2**:343-369. 1942.
6. FELDMAN, W. H., and OLSON, C., Jr. Neoplastic Diseases of the Chicken. *In Diseases of Poultry*, edited by H. E. Biester. Ames: Iowa State College Press, **30**:523-597. 1943.
7. FURTH, J. Private communication.
8. GREENE, H. S. N. Familial Mammary Tumors in the Rabbit. IV. The Evolution of Autonomy in the Course of Tumor Development as Indicated by Transplantation Experiments. *J. Exper. Med.*, **71**:305-324. 1940.
9. JUNGHERR, E. Studies on Fowl Paralysis. 2. Transmission Experiments. *Storrs Agric. Exper. Sta. Bull.*, **218**:1-47. 1937.
10. KING, J. W., and DURAN-REYNALS, F. Development by Ducks of Natural Neutralizing Antibodies for a Duck Variant of the Rous Sarcoma Virus. *Yale J. Biol. & Med.*, **16**:53-57. 1943.
11. OLSON, C., JR., and BULLIS, K. L. A Survey and Study of Spontaneous Neoplastic Diseases in Chickens. *Mass. Agric. Exper. Sta. Bull.*
12. REIS, J., and NOBREGA, P. *Tratado de Doenças das Aves*. Edicao do Instituto Biologico. Sao Paulo, Brazil, 1935.
13. SHRIGLEY, E. W., GREENE, H. S. N., DURAN-REYNALS, F. Studies on the Variation of the Rous Sarcoma Virus Following Growth of the Tumor in the Anterior Chamber of the Guinea Pig Eye. *Cancer Research*, **5**:356-364. 1945.
14. TROISIER, J. Etude expérimentale de la sarcomatose spontanée des poules. *Bull. Assoc. franç. p. l'étude d. cancer*, **23**:225-270. 1934.