UTERINE ADENOMATA IN THE RABBIT

III. Susceptibility as a Function of Constitutional Factors

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Adenomatous tumors of the uterine fundus are generally believed to be the most common form of neoplasia in the rabbit, and in a recent review Polson found reference to 29 instances and noted that approximately 50 per cent of the tumors reported in the literature were of this type (1). However, the majority of recorded instances represent accidental findings and available data give no indication of the actual incidence of such tumors in rabbit populations. A study of the tumors was undertaken in this laboratory as part of a broad investigation of spontaneous neoplasia in the rabbit, and an account of their clinical history, pathology, and incidence up to 1938 has been reported (2). The high incidence has continued and a further, more detailed report seems desirable both because of the increased use of the rabbit in experimental cancer research and because of the constitutional implication arising from the study.

The investigation originated and has been continued as a population survey. No attempt has been made to increase the frequency of tumors by selective breeding and there is no reason to believe that the high incidence is peculiar to our colony and not representative of other rabbit populations made up of similar constitutional types. However, the colony in question differs from other rabbit populations in that it is of known composition and the behavior of various stocks and individual animals has been the subject of extensive study. Because of this, it has been possible to analyze the incidence in relation to various constitutional factors and thus to investigate the bearing of inherent constitutional peculiarities on the genesis of the growths. The object of the present paper is to present an analysis of the incidence of the tumors from this point of view. It should be emphasized, however, that the study was carried out as part of a broad constitutional investigation involving a great many diverse problems. Animals could not be held under observation for unlimited periods of time without the abandonment of equally important lines of investigation and it

was necessary to pursue the study in coordination with other problems rather than as an isolated or independent investigation.

Materials and Methods

The organization and management of the colony have been described in detail elsewhere (3). It should be noted, however, that the colony is maintained in active breeding service and the pedigrees and life histories of all animals are known.

During the period of this investigation, the population was made up of 14 pure breeds, including the Belgian, Beveren, Chinchilla, Dutch, English, Havana, Himalayan, Polish, Rex, Sable and Silver Marten, Siamese Sable, French Silver, and Tan breeds, and numerous hybrid lines. Several of the hybrid lines have been carried without the introduction of foreign blood for a considerable number of generations and are referred to in the text as pure lines. Other lines derived primarily from a cross between two pure breeds have been maintained by repeated breeding with one of the parental lines and are denoted as hybrid concentrates. The majority of other hybrids constitute F_1, F_2, or backcross generations of the various pure breeds but a small number represent multiple pure breed and hybrid crosses and are referred to as complex polyhybrids.

The present report is based on the incidence of tumors in this population between September, 1931, and February, 1940. During this period 145 tumor-bearing animals have come under observation. With a single exception, all of the growths were found after the 2nd year of life and for present purposes only animals beyond this age will be considered in statistical studies. Three of the tumors occurred in rabbits obtained from outside sources. One of these was pure bred and its age and ancestry were known, but in the other instances neither age nor pedigree was obtainable and they are therefore not included in the following analysis. The material to be used for statistical analysis thus consists of 142 instances of tumor found among 849 animals more than 2 years of age.

The majority of tumors were first discovered during the course of routine examination of the uterus during life and, in all instances, their presence was confirmed by biopsy or autopsy within the following 2 weeks. The tumors can be palpated in the uterus when they have attained a diameter of 0.5 cm., and it should be pointed out that the ages used throughout the report represent this stage of development rather than the beginning of the neoplastic process.

It was not possible to carry all animals bred in the colony to an age of 2 years and selection was made on a basis of their utility in the investigation of other constitutional problems. These problems were concerned with abnormal constitutional types and many of the animals held were abnormal in some respect or were known to transmit some type of physical or functional variation. However, in our experience all rabbits subjected to sufficient inbreeding and study become classifiable in the latter group and it should not be assumed that the stocks were a more abnormal population than those of other colonies would be if handled in like manner. The difference lies in the fact that in the colony in question the abnormal animals were known and classified.

Age

The incidence of tumors in relation to age is presented in Table I. It will be observed that the incidence increased from 4.2 per cent in animals

2 to 3 years old to 79.1 per cent in animals 5 to 6 years old. It should be emphasized that these figures represent new tumors occurring at the stated periods and do not indicate the total number of growths which might be found in a population of a given age. Figures obtained in such a manner would obviously represent tumors at different stages of development and their value would depend upon the length of life of the tumor bearing animals.

The incidence of tumor development increased with age, and it is apparent from examination of the table that if a rabbit survived to the 5th year of life without the occurrence of a uterine tumor,—which is contrary to probability,—the chances are better than 3 to 1 that a tumor would develop in the 7th year.

TABLE I

The Incidence of Uterine Tumors in Relation to Age

	Age at discovery of uterine tumor					
	2–3 yrs.	3–4 yrs.	4–5 yrs.	5–6 yrs.	6–7 yrs.	Total
Number of rabbits	491	259	71	24	4	849
Number bearing uterine tumors	21	54	45	19	3	142
Incidence of uterine tumors, *per cent*	4.2	20.8	63.3	79.1	75.0	16.7

Breed

The incidence of tumors in the various elements of the population is presented in Table II. In this and in subsequent tables data are presented in relation to age as well as to the constitutional factor under consideration, for it is obvious in view of the pronounced differential age susceptibility that the incidence in a given constitutional group depends in part upon the age distribution of the animals.

Examination of the table shows that only a slight variation in incidence distinguishes pure breeds, pure lines, and hybrid concentrates, while considerable variation occurs between several of the hybrid classes. The pure breeds, pure lines, and concentrates are made up of extremely diverse constitutional types and the only factor held in common is the factor of relative homogeneity. On the other hand, heterogeneity is the dominant characteristic of hybrids. Apparently, therefore, there exists a relationship between homogeneity and susceptibility to neoplasia, and the variation in incidence from 14.2 per cent in inbred or comparatively homogeneous stocks to 21.1 per cent in hybrids would suggest that heterogeneity favored tumor development.

Pure Breeds.—No instances of the tumor occurred in the Belgian or Rex

breeds and the arrangement of breeds in order of increasing incidence stands, Polish, Himalayan, Sable, Beveren, Chinchilla, English, Marten, Dutch, Havana, French Silver, and Tan (Table III). The latter two breeds were represented by few animals and the high incidence is of doubtful significance.

A consideration of the racial relationships of the various breeds brings out a number of points of interest. The Rex breed is distinguished from other rabbits by a short haired coat character. This character has been utilized in the development of "rex" varieties of a number of other breeds

TABLE II

The Incidence of Uterine Tumors in Various Elements of the Population

	Age at discovery of uterine tumor											
	2–3 yrs.		3–4 yrs.		4–5 yrs.		5–6 yrs.		6–7 yrs.		Total	
	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
Pure breeds....	192	4.1	81	22.2	26	38.4	14	71.4	2	50.0	315	14.9
Pure lines.....	23	4.3	20	15.0	4	50.0	0		0		47	12.7
Hybrid concentrates.....	107	3.7	62	17.7	7	85.7	4	75.0	0		180	13.3
Total.....	322	4.0	163	19.6	37	48.6	18	72.2	2	50.0	542	14.2
Backcross hybrids.....	33	6.0	16	43.7	5	60.0	0		0		54	22.2
F_2 hybrids.....	10	10.0	2	0	1	100.0	0		0		13	15.3
F_1 hybrids.....	113	3.5	75	20.0	25	80.0	6	100.0	2	100.0	221	21.2
Complex polyhybrids.....	13	7.6	3	0	3	100.0					19	21.0
Total.....	169	4.7	96	22.9	34	79.4	6	100.0	2	100.0	307	21.1

so that at present the term possesses no racial significance. Our Rex stock was derived from the original Gillet strain and from the beginning has shown unmistakable evidence of a Belgian origin. It is not surprising, therefore, that the absence of tumors which distinguished the Belgian breed should also characterize the racially related Rexes.

The Dutch, Polish, Havanas, and Tans are also racially related and belong to a group which is fundamentally Dutch. With the exception of the Polish these breeds ranked highest in susceptibility. The Chinchillas, Sables, Martens, and Himalayans are members of the albino series; all are related to the Himalayans and the Martens are related to the Tans. The group as a whole occupied an intermediate position in the susceptibility scale and the various breeds showed little variation in incidence but, as

would be expected in view of the Tan relationship, the Martens ranked highest. The English, Beverens, and French Silvers are racially independent and no pure bred relatives were present in the colony.

The pure breeds of the colony were operated under a plan of breeding which disposed toward homogeneity and with a single exception no attempt was made to maintain individual strains. The exception is represented by the English breed which was carried for a long time as two separated strains, together with a third element made up of strain crosses. One of the strains

TABLE III

The Incidence of Uterine Tumors in Relation to Breed

	Age at discovery of uterine tumor											
	2–3 yrs.		3–4 yrs.		4–5 yrs.		5–6 yrs.		6–7 yrs.		Total	
	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
Belgian	28	0	10	0	3	0					41	0
Beveren	9	0	2	50.0	2	50.0					13	15.3
Chinchilla	6	16.6	3	33.3	2	0	1	0			12	16.6
Dutch	25	4.0	8	50.0	1	100.0	1	100.0			35	20.0
English	38	7.8	10	30.0	6	16.6	2	100.0	2	50.0	58	17.2
Havana	30	3.3	20	25.0	6	66.6	4	75.0			60	21.6
Himalayan	16	0	8	12.5	4	50.0	2	0			30	10.0
Marten	13	0	8	25.0	1	100.0	1	100.0			23	17.3
Polish	9	0	3	33.3							12	8.3
Rex	4	0	5	0	1	0					10	0
Sable	9	0	2	0			2	100.0			13	15.3
French Silver	3	33.3	1	0							4	25.0
Tan	2	50.0	1	0			1	100.0			4	50.0
AA	6	0	5	40.0							11	18.1
HAA	17	5.8	15	6.6	4	50.0					36	11.1

represented the nearest approach to normal that has been encountered in the rabbit. 19 animals of this line were carried to the 2nd year of life and 8 survived the 3rd year with an incidence of 5.2 per cent of tumors in the whole group. The drop in tumor incidence observed in the 4 to 5 year age period of the English in Table III in contrast to an increase in other breeds is accounted for by the death of many animals of this strain at that age period. The other strain presented many abnormalities, including transmission of a dwarf variation, and carried a variety of functional disturbances of such severity that, while 20 animals were held through the 2nd year of life, only 2 survived the 3rd year. The incidence of tumors in this strain was 10 per cent but it seems probable that the incidence would have been

greatly increased if it had been possible to carry animals to a more susceptible age. A more viable group was obtained from crosses between the two strains and 20 such animals were carried to the 2nd year of life and 11 survived the 3rd year. The incidence of tumors in the group was 35 per cent and it is in line with other findings to assume that the increased susceptibility was related to the factor of heterogeneity which formed the contrasting genetic feature of this group in relation to the two pure strains.

Individual strains of other breeds have been carried without the introduction of new blood for variable periods of time but, in most instances, deterioration occurred and the strains were either merged to preserve the breed or dropped. However, both the Himalayan and Havana breeds have been carried as pure lines for 10 and 11 years respectively. The Himalayan breed has recently been discarded because of sterility but the Havana line can still be bred. The point of interest in the present connection lies in the fact that the two breeds represent a maximum of homogeneity and, in view of the wide variation in tumor incidence, 10 per cent and 21 per cent, it would appear that race rather than the relative degree of inbreeding was the dominant factor in the determination of breed susceptibility.

Pure Lines.—Two hybrid lines designated in Table III as "AA" and "HAA" were carried without the introduction of foreign blood for a long series of generations and represent an approach to pure lines. The "AA" line was founded in 1918 on a mongrel stock of uncertain origin derived in part from a Dutch-Polish cross. The incidence of tumors was intermediate in relation to these breeds but approached the higher figure observed in the Dutch. The "HAA" line was derived primarily from a cross of the "AA" line with pure bred Himalayans and the decreased incidence of tumors is apparently referable to the influence of Himalayan factors.

Hybrid Concentrates.—The incidence of tumors in the various hybrid concentrates is presented in Table IV. It should be emphasized that these animals represent continued breeding back to the parental line indicated in the table with extreme dilution of the extraneous blood introduced in the primary outcross. Despite such dilution, it is apparent from examination of the table that in a number of instances the introduced factors exerted a profound effect on the constitution of the animals.

The incidence in the Dutch, Beveren, and Rex breeds was little influenced by the outcross and the susceptibility of the Silvers and English was apparently decreased. However, with respect to the latter findings, it should be noted that the incidence observed in the pure bred Silvers is of doubtful significance because of the small number of animals and that the concentration of English factors was generally in the direction of the less sus-

ceptible strain. In the case of other breeds, on the contrary, the incidence was much increased. This is especially notable in the Belgian concentrates in which the dilution of foreign blood was even greater than in other instances, but in which the incidence was increased from 0 to 7 per cent. In like manner, the incidence in the Havanas was increased from 21.6 per cent to 33.3 per cent, the Polish incidence rose from 8.3 per cent to 19 per cent and the incidence in the "HAA" line increased from 11.1 per cent to 20.5 per cent. It is suggested that these results represent additional evidence of the influence of heterogeneity and indicate that deviations from homogeneity, even of the minor degree present in these cases, are sufficient to

TABLE IV

The Incidence of Uterine Tumors in Hybrid Concentrates

Direction of concentration	Age at discovery of uterine tumor									
	2–3 yrs.		3–4 yrs.		4–5 yrs.		5–6 yrs.		Total	
	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors
		per cent		*per cent*		*per cent*		*per cent*		*per cent*
Belgian	34	2.9	22	9.0	1	100.0			57	7.0
Beveren	7	0	1	100.0					8	12.5
Dutch	16	6.2	8	12.5	3	100.0	2	50.0	29	20.6
English	6	0	2	0					8	0
Havana	3	33.3							3	33.3
Polish	11	0	7	14.2	1	100.0	2	100.0	21	19.0
Rex	9	0	4	0					13	0
French Silver	5	20.0	2	0					7	14.2
HAA	16	0	16	37.5	2	50.0			34	20.5

produce marked alterations in constitution and in the susceptibility to tumor development.

Hybrids.—Table V contains an analysis of the incidence in first, second, and backcross hybrids of all age groups beyond the 2nd year. The material differs from that presented in the latter part of Table II and for purposes of simplification only true breed crosses are considered, whereas Table II includes crosses between unrelated pure lines and unrelated hybrids as well as the different generations derived from them. The total incidence and the incidence in various age periods as shown in Table II were not significantly changed by this selection of material.

An interpretation of the variations in incidence from the point of view of breed factors or of heterogeneity presents a complicated problem. The majority of pure bred animals selected for hybridization were abnormal in some respect or were known to transmit variations of a detrimental nature

and their progeny were bred expressly for the study of these abnormalities. As a result, a large part of the hybrid stocks showed or transmitted constitutional variations and the influence of these variations on tumor incidence complicates an evaluation of the significance of other factors.

It will be noted that the incidence in the first hybrid generation was increased in the majority of cases. The number of animals in the second generation was generally too small for significant analysis but, as a rule, the incidence was approximately of the same order as that observed in the pure breeds. The backcross generation was also small but in the majority

TABLE V

The Incidence of Uterine Tumors in F_1, F_2, and Backcross Hybrids

	F_1 generation		F_2 generation		Backcross generation		Total	
	Number	Incidence	Number	Incidence	Number	Incidence	Number	Incidence
		per cent		*per cent*		*per cent*		*per cent*
Belgian	33	9.0	1	0	6	0	40	7.5
Beveren	8	25.0			7	42.8	15	33.3
Chinchilla	11	9.0	1	0			12	8.3
Dutch	25	24.0	5	20.0	7	42.8	37	27.0
English	59	28.8	2	0	4	0	65	26.1
Havana	48	22.9	5	20.0	4	0	57	21.0
Himalayan	21	33.3	1	0	2	50.0	24	33.3
Marten	5	0					5	0
Polish	49	42.8	6	16.6	6	16.6	61	37.7
Rex	18	5.5	1	0	13	23.0	32	12.3
Sable	1	0					1	0
Silver	7	0	2	50.0	1	0	10	10.0
Tan	7	14.2			3	33.3	10	20.0

of groups containing sufficient animals the incidence was very much increased.

The increased incidence in the F_1 generation with the return to the general level of pure breeds in the F_2 generation is in line with other observations indicating a relationship between heterogeneity and increased susceptibility. It is of interest to note in this connection that the two F_1 groups that showed the slightest increase over pure breed incidence, namely the Dutch and Havanas, were derived almost entirely from hybrid matings within their own family of breeds. Moreover, the apparent decrease in incidence in the case of the Silvers and Tans is without significance inasmuch as the actual incidence in pure bred animals of these types is not known.

The high incidence in the backcross generation would appear to contradict

the existence of a direct relationship between heterogeneity and susceptibility, for such animals constitute a closer approach to the status of the parent pure breed than do F_1's, yet the incidence in this group was generally greater than in the F_1 generation. It seems probable, however, that other influences were concerned in the determination of the high susceptibility of this generation and the evidence at hand indicates that abnormal constitutional factors incorporated in the stock were of great significance in this respect. Accordingly, a higher incidence of tumors would be expected in the backcross generation which represented a concentration of such factors than in the F_1 generation in which the factors were present only in heterozygous form. Evidence relating to the influence of constitutional abnormalities of this nature will be presented in the following paragraphs.

Constitutional Variation

Examination of Table V shows that the highest incidence in the various hybrid groups occurred in the Polish. The animals of this group had been bred largely for study of an hereditary variation characterized by a dwarfing effect which in homozygous form is lethal and produces a miniature individual approximately one-third the size of its normal sibs. Heterozygous animals are approximately two-thirds the weight of their normal sibs, never attain an equal stature, and are subject to a variety of functional disorders (4).

124 animals derived from the dwarf transmitting Polish line by a variety of hybrid matings were thoroughly tested and were held under observation for 2 or more years. 58 of the animals were found to transmit the variation and the incidence of tumors in this group was 48.2 per cent in contrast to an incidence of 18.1 per cent in non-transmitting animals of the same derivation or of 14 per cent in the remainder of the population (Table VI).

In like manner, a large proportion of the Dutch hybrids were bred for study of a constitutional abnormality which, because of the character of its dominant manifestations, has been referred to as cretinoid (5). This abnormality is apparently a complex or composite character and its different features may be transmitted and inherited independently, while the variation is expressed in typical form only when certain component parts are recombined in an individual. Thus, it was not possible, as in the case of the dwarf variation, to classify the animals into clean cut divisions of transmitters and non-transmitters, and for present purposes all animals known to transmit any feature of the syndrome have been grouped together and designated as the cretinoid line.

Examination of Table VII shows that 33.7 per cent of the 83 animals of

this line held for 2 or more years developed tumors of the uterus. However, approximately one-half of these animals were obtained by cross breeding with the Polish dwarf line and many were heterozygous for the factors concerned in the dwarf variation. The question, therefore, arises as to whether the high susceptibility was a function of the cretinoid complex or was related to the incorporation of the dwarf variation in the line. Table VII presents an analysis of the incidence based on this question and brings out a number of points pertaining to the relationship of constitutional variation and susceptibility.

The incidence of tumors in the cretinoid line of non-dwarf derivation was almost identical with that in the line obtained from cross breeding with

TABLE VI

The Incidence of Uterine Tumors among Transmitters of the Dwarf Variation

	Age at discovery of uterine tumor											
	2–3 yrs.		3–4 yrs.		4–5 yrs.		5–6 yrs.		6–7 yrs.		Total	
	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
Dwarf transmitters.	23	4.3	15	53.3	13	92.3	7	100.0			58	48.2
Non-dwarf transmitters of the same derivation.....	43	6.9	15	20.0	5	80.0	2	50.0	1	100.0	66	18.1
Non-dwarf transmitters of different derivation.....	425	4.0	229	18.7	53	54.7	15	73.3	3	66.6	725	14.0

dwarf stock and it follows that the factors concerned in the cretinoid variation were themselves determiners of the high susceptibility of that line. It might be expected from this that the crossing of the dwarf and cretinoid lines, both of which carried factors favoring tumor development, would result in the production of animals of higher susceptibility than was shown by either of the parental lines. However, this did not occur and the same incidence characterized transmitters and non-transmitters of the dwarf variation irrespective of their derivation; that is, the susceptibility of animals carrying both the dwarf and cretinoid variations was not greater than that of animals carrying the dwarf variation alone.

An additional point requiring further investigation is the pronounced difference in incidence observed between non-dwarf transmitting animals of the cretinoid-dwarf line and animals of the cretinoid line derived from non-dwarf stock. Presumably, these classes were genetically similar as

regards the dwarf and cretinoid variations, yet in the former class the incidence was 18.1 per cent in contrast to an incidence of 34.1 per cent in the latter.

The relationship between tumor development and constitutional variation was most clearly defined in the case of the dwarf and cretinoid variations but was also apparent in other abnormal types. However, other constitutional types have not been so thoroughly studied or are of such

TABLE VII

A Comparison of the Incidence of Uterine Tumors in the Cretinoid Line and Its Various Subdivisions with the Incidence in the Pure Dwarf Line

	Age at discovery of uterine tumor											
	2–3 yrs.		3–4 yrs.		4–5 yrs.		5–6 yrs.		6–7 yrs.		Total	
	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors	Rabbits	Tumors
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
Pure dwarf line												
Dwarf transmitters	16	6.2	12	58.3	8	100.0	2	100.0			38	47.3
Non-dwarf transmitters	32	6.2	7	28.5	4	75.0			1	100.0	44	18.4
Cretinoid dwarf line												
Dwarf transmitters	7	0	3	33.3	5	80.0	5	100.0			20	50.0
Non-dwarf transmitters	11	9.0	8	12.5	1	100.0	2	50.0			22	18.1
Total	18	5.5	11	18.1	6	83.3	7	85.7			42	33.3
Cretinoid non-dwarf line	22	9.0	14	50.0	3	100.0	2	100.0			41	34.1
Cretinoid line total	40	7.5	25	31.0	9	88.8	9	88.8			83	33.7

complex nature that clearly defined classification into categories of sufficient size to allow a significant differential analysis of the tumor incidence has not been possible so far.

The high tumor incidence which characterized transmitters of the Polish dwarf variation was also associated with transmission of other types of dwarfism. The presence of a dwarf variation in the more susceptible strain of the English breed has been mentioned and other dwarf forms occurred with high frequency in the Himalayans and Havanas. A type of dwarfism was transmitted by 2 of the tumor bearing animals derived from the Belgian breed, while the remaining 5 tumor bearing animals of this category trans-

mitted a complex constitutional variation frequently associated with a diminution in size. Thus, heterozygosity for some form of dwarfism was the most constant genetic peculiarity of tumor bearing animals, but the transmission of a multiplicity of variations ranging from physical anomalies such as hydrocephalus, brachydactylia, and pterygium to extremely complex functional syndromes characterized the constitutional status of others and, in general, it may be said that the tumors occurred in that portion of the population classed as abnormal rather than in the so called normal groups.

Parent-Progeny Relations

It is clear from the preceding sections that many of the tumor bearing animals were racially or constitutionally related and a consideration of this relationship on a parent-progeny basis brings out a number of points of interest.

The degree of interrelationship within the group is indicated by the fact that all 142 tumor bearing animals were derived from the matings of 62 males and 103 females. Tumors occurred in mothers and daughters in 19 instances. In several cases 4 to 5 daughters of tumor bearing animals were affected so that, considering the group as a whole, tumors developed in 34 daughters of 19 tumor bearing mothers. Moreover, there were 3 instances in which tumors occurred in mothers, daughters, and granddaughters and in one instance 4 granddaughters were affected.

Significant conclusions cannot be drawn from an analysis of different classes of progeny based on parental tumor relations, for many of the parents as well as progeny died or were discarded at an early age or are still living and their ultimate fate is unknown. However, despite unavoidable errors of classification and the impossibility of accurate interpretation the results as they stand at present are of some interest.

A total of 148 progeny of tumor bearing animals survived for 2 or more years and 34 of these developed tumors. This is an approximation of 1:3 ratio as might be expected in the backcross generation of a two factor variation. However, if such a ratio is accepted it must be assumed that all of the males used were heterozygous for the variation, and this appears improbable. On the other hand, if only matings with males whose progeny bore tumors are considered, there remain 85 animals of which 34 were tumor bearing and this may represent either a 1:1 or 1:2 ratio.

A consideration of other possible tumor generations does not aid in clarifying this question. If the remainder of the population is considered as arising from F_2 tumor matings, one obtains a ratio of 701 to 108 which is without genetic significance. On the other hand, if only matings between

males and females whose progeny bore tumors are considered as F_2's, there occurs a ratio of 179 to 108 which shows far too great a preponderance of affected individuals for an F_2 class.

It is clear from these results that many of the animals in whose progeny tumors occurred would themselves have developed tumors if they had been held under observation for a longer period of time and, because of this, an accurate classification of parents and progeny for purposes of genetic analysis is obviously impossible. While an interpretation of the ratios derived from classifications constructed on available facts gives no indication of the actual mode of inheritance, at the same time the results obtained leave no doubt of the existence of a hereditary factor in tumor susceptibility.

Relation to Disorders of Pregnancy

In the first paper of this series attention was called to the occurrence of abnormalities in the breeding behavior of animals that subsequently developed tumors of the uterus and, because of their frequency, it was suggested that they formed an integral part of the clinical history of the tumors (2). The abnormalities have occurred, with few exceptions, in the additional cases reported in the present paper and an attempt has been made to determine their relationship to tumor development.

In general, the early breeding history of the animals is normal in all respects, and abnormalities are usually not apparent until the third or fourth gestation or may be delayed to a much later pregnancy. The first disturbance may occur as a frank attack of toxemia of pregnancy but more often the manifestations are those of slight illness of indeterminate type occurring during the last week of gestation. In many instances there are no further evidences of disorder, but frequently the resulting litter contains still born young or is born dead in entirety. In the former case, the occurrence of early deaths resulting from inadequate nutrition suggests that the maternal disorder continued after the birth of the litter. Other attacks of this type may recur in milder or more aggravated form toward the end of a later gestation but intervening pregnancies may be entirely normal. In other cases, no further disturbance of this nature is noted but eventually in all instances there occurs a gradual diminution of the fertility rate with a reduction of the number of young per litter. This may continue to the point of complete sterility before the presence of tumor in the uterus is detected, but in several instances it has been found that following multiple matings repeated at frequent intervals, animals bearing small early tumors may become pregnant and after a normal gestation period give birth to 1 or 2 young.

An investigation of this poorly defined disorder of pregnancy was under-

taken primarily in an attempt to determine its relationship to toxemia of pregnancy which was known to occur with high frequency in the same line of animals. A considerable number of animals displaying signs of the disorder were subjected to chemical blood study and a number of these were subsequently killed for histological examination. In brief, it was found that the chemical blood alterations and tissue changes were identical in type with those observed in fatal cases of toxemia of pregnancy and it was concluded that the disorder under consideration represented a less acute disturbance of the same nature (6).

The blood of several of the tumor bearing animals included in the present report was studied during attacks of the disorder and typical changes were found but, in other cases, chemical analyses were not performed and the only evidence that these animals suffered mild attacks of toxemia of pregnancy lies in the occurrence of disturbances of the nature described.

It is reasonable to assume, if toxemia of pregnancy and the development of uterine tumors are as intimately associated as these observations indicate, that the same differential susceptibility would occur in relation to breed and other constitutional factors. A determination of the actual incidence of toxemia of pregnancy, including the milder types with obscure clinical signs, has been beyond the scope of our facilities and the only accurate data that can be used for comparative purposes pertain to fatal cases of the disorder. These have been reported in detail and need not be repeated here (7). It is sufficient to note that with few variations in position, the standing of the various breeds in susceptibility scales was essentially the the same with the highest incidence of both disorders occurring in the Dutch group and the lowest in the Belgians. Moreover, the incidence of toxemia of pregnancy as well as of uterine tumors was highest in first generation hybrids and in constitutional groups characterized by transmission of the dwarf and cretinoid abnormalities.

Confirmatory evidence of the close association of the two disorders lies in the high incidence of fatal toxemia among the immediate non-tumor bearing relatives of tumor animals. There were 132 animals of this class, representing sisters or daughters of tumor bearing females derived from matings with males whose progeny were known to have developed tumors of this type. 35 of these animals were killed because of persistent infertility and 40 were disposed of for other reasons. But, of the remaining 57, 28 or 49.1 per cent died of toxemia of pregnancy.

It is of interest in this connection to note that all of the 35 females killed because of infertility showed pronounced cystic endometritis and endocrinological changes similar to those found in tumor bearing animals.

Successive biopsy examinations performed on a large series of rabbits have shown that cystic endometritis of this type represents an early stage of tumor development and forms a constant feature of the period of decreasing fertility that characterizes the breeding history of tumor animals. It seems probable, therefore, that typical tumors would have developed if these animals had been held under observation for a longer period of time.

DISCUSSION

The investigation described in this paper brings out two main points which warrant emphasis. First, uterine tumors occur with a high incidence in rabbits and, as will appear, their anatomical distribution is in direct contrast to that found in women. Second, the incidence of the tumors in the rabbit is highly selective and susceptible animals are marked by distinctive constitutional traits.

The high incidence of the tumors is of immediate importance in view of the increased use of the rabbit as an experimental animal in cancer research, and recent reports in the literature ascribing their occurrence to experimental procedures must be considered in the light of this finding. The high incidence is not related to factors peculiar to this laboratory, for the growths have been found in a high proportion of animals imported from outside breeding colonies. It is apparent, therefore, that research concerned with the induction of tumors of this type must be stringently controlled with respect to the age, breed, and genetic qualities of test animals and that the use of animals of unknown constitution must preclude any attempt to estimate the significance of experimental results.

All of the tumors referred to in this report originated in the endometrium of the uterine fundus and, despite the postmortem study of 849 rabbits 2 or more years old, no instance of cervical cancer has been seen. The significance of this statement is enhanced by the fact that the rabbit's uterus is bicornate and contains two cervices. Thus, nearly 1700 cervices have been examined without the discovery of a single tumor. Moreover, a review of the literature yields no instance of cervical cancer in this species. On the other hand, more than 90 per cent of uterine tumors in women originate in the cervix and fundus cancers are comparatively infrequent. The finding is therefore of interest from the point of view of cervical growths in women as well as of fundus tumors in the rabbit and leads to a consideration of differences in anatomical structure and physiological behavior as possible factors in the determination of the variation in incidence.

The human cervix is characterized by the possession of two different types of epithelia. The cervical canal is lined by columnar epithelium, while the

covering of the portio vaginalis is continuous with that of the vagina and consists of stratified squamous epithelium. An abrupt junction occurs just outside the external os and creates a region particularly susceptible to cell disturbances. The junction is not fixed and the epithelia are readily interchangeable throughout different age periods or in response to pathological stimuli such as trauma or inflammation. This peculiar epithelial instability in combination with the physiological activity of the part and the inflammation and trauma to which it is exposed are generally accepted as important factors in the etiology of cervical cancer.

In contrast, the epithelium of the rabbit's cervix is columnar throughout. The junction with stratified squamous epithelium occurs in the region of the urethral meatus at the upper border of the true vulva and the columnar lining is continuous from the uterine cavity to this point. The great majority of human cervical cancers are of the common epidermoid variety arising from squamous epithelium and the absence of this epithelium in the rabbits' cervix accounts in large part for the absence of cervical carcinomata in the species. The lack of adenocarcinomata, which form less than 4 per cent of cervical growths in women, may in turn be related to the more stable constitution of the columnar epithelium which prevails in the absence of the antagonistic stimulation of adjacent squamous epithelium. Thus, this finding not only tends to account for the variation in the incidence of cervical tumors in the two species but also places strong emphasis on the importance of the epithelial junction in the genesis of cervical neoplasia in women.

At the same time, the variation in the frequency of fundus cancers remains unexplained. Experimental investigations indicate that a common endocrinological factor is operative in the genesis of both cervical and fundus tumors and, inasmuch as cervical growths occur at an earlier age period in women, it is conceivable that the instable epithelial junction determines a point of greatest uterine susceptibility and the consequent development of cancer inhibits later expression of the factor in the fundus. In the rabbit the absence of the epithelial junction would eliminate the cervix as a point of least resistance and allow expression of the endocrinological factor in regions of greater susceptibility.

Special features of the endometrium of the fundus in the rabbit would appear to increase its susceptibility over that of other uterine regions and the occurrence of multiple pregnancies repeated at frequent intervals may be of particular significance in this respect. Some association with placentation is clearly indicated by the distribution of the tumors which are usually multiple and arise from the placental folds in spaced arrangement,

simulating the disposition of placentae in the gravid uterus. The manner of placentation in the rabbit would not of itself seem to constitute a more predisposing factor than the analogous process in women. A more pertinent feature in this respect appears to be the frequent repetition of placenta formation which, moreover, is continued in the rabbit beyond an age period which would correspond to the menopause in women. It is therefore suggested that, just as the presence of the epithelial junction creates a site of maximum susceptibility in the cervix of women, so also the augmented physiological activity of the fundus epithelium in the rabbit with the consequent increased liability to altered cellular relations renders this tissue the most susceptible uterine region to the action of the specific endocrinological factor.

Local susceptibility thus appears to bear a direct relationship to the anatomy and physiology of the part, but inasmuch as the endometria of all breeding rabbits share the susceptibility and the incidence of tumors remains highly selective, it is apparent that other factors are concerned in the genesis of the tumors. Consideration of this aspect of the problem leads to the second point raised for discussion and indicates an examination of the constitutional traits that distinguish susceptible animals and the possible bearing of such traits on the genesis of the growths.

Certain lines and groups of animals are highly susceptible while the tumors rarely or never occur in others, and analysis shows that breed and genetic constitution are the distinguishing features. However, the rôle in tumor genesis played by the genetic factors concerned in breed determination or in the peculiarities of susceptible animals is not immediately apparent and the relationship between susceptibility to neoplasia of the uterus and the factors that distinguish Dutch from Belgian rabbits or determine the occurrence of the dwarf variation is not direct but rather appears to depend on a causal sequence in which intermediate factors are of great importance.

The close parallelism between the incidence of toxemia of pregnancy and of uterine tumors is of significance in this respect. Analysis of the incidence of toxemia of pregnancy shows a similar frequency distribution with the same breed and constitutional relationships observed in the case of the tumors, and the position of different genetic groups in a susceptibility scale is identical with respect to both disorders. Moreover, there is definite evidence that all of the tumor bearing animals suffered mild attacks of toxemia during earlier life.

The mild form of toxemia of pregnancy has been extensively studied and the same chemical and pathological changes which characterize the fatal

disorder have been found. The histological changes are most pronounced in the liver and suprarenal, while the kidney damage is much less intense. The alterations in blood chemistry and the organ damage persist for a long period of time after apparent clinical recovery and in one instance the chemical blood values were still abnormal when determinations were made a year later. The point to be emphasized is that all of the tumor bearing animals suffered intense liver injury during earlier life. There is anatomical as well as chemical evidence that the injury persisted for long periods of time and, despite the final restoration of an apparently normal histological structure, it is conceivable that certain non-vital functions may have been irrevocably altered and that complete physiological recovery never occurred.

This point brings the problem into relation with known facts. Zondek in 1934 found that liver mash inactivated estrogen *in vitro*, while mash from other organs was ineffective (8). Later Israel, Meranze, and Johnstone showed that estrin was inactivated in a heart-lung-liver perfusion system, whereas no inactivation occurred if the liver was withdrawn from the system (9). Again in 1938, Golden and Severinghaus in an ingenious homotransplantation experiment showed that if the secretions of rat ovaries passed directly to the uterus, vagina, and pituitary, estrus recurred shortly after operation but, on the contrary, if the secretions passed through the liver before reaching other organs, the estrus cycle was not resumed (10). Finally Talbot in 1939 damaged the livers of intact female rats with a mixture of carbon tetrachloride and alcohol and observed the uterine response that characterizes an increased concentration of estrogen in the circulation (11). Thus, experimental evidence places the liver as the principal organ of the body concerned with the inactivation of estrogen and shows that damage (fatty degeneration, focal necrosis) renders it incapable of performing this function.

The liver lesions which characterize toxemia of pregnancy are almost identical with those produced by poisons such as carbon tetrachloride and practical distinction is based on clinical history rather than on anatomical changes. It would appear, therefore, that rabbits with liver damage of that nature would be subjected to an increase concentration of estrogen and, if the damage persisted as in non-fatal cases of the disorder, the concentration would eventually rise to a carcinogenic level.

Abundant evidence that estrin represents the exciting agent in the genesis of the uterine tumors has been presented in other papers (2) and, in view of the absence of indicative ovarian changes, it is suggested that the incapacity of the liver to inactivate a normal secretion of the substance

rather than a primary ovarian hypersecretion forms the basis of its action. This suggestion has been investigated and, while the experiments have not been published and are being continued, the results bear directly on the point in question and will be cited in the present context. Non-fatal attacks of toxemia were induced in a group of 6 month old rabbits (12) and small doses of estrin were administered weekly throughout the following 3 months. A control group of normal, untreated animals of the same age received a corresponding amount of estrin over the same period of time. The endometrium of animals subjected to preliminary liver damage was extremely irregular with pronounced cystic degeneration and small areas resembling early tumor formation, whereas that of control rabbits receiving estrin alone showed only a moderate hyperplasia.

Apparently, therefore, the constitutional relationships observed in a study of the incidence of uterine tumors rest primarily on susceptibility to toxemia of pregnancy and gain expression through this circumstance rather than through the action of independent determining factors. The factors controlling susceptibility to toxemia of pregnancy are evidently endocrinological in nature. This disorder appears to be of hypophyseal origin (7) and the high incidence of cases in animals with known endocrine abnormalities (4, 7) suggests that the apparently diverse factors associated with susceptibility might eventually all be resolved into variations in endocrinological constitution.

SUMMARY

An investigation of uterine adenomata in the rabbit revealed an incidence of 16.7 per cent in the total female population over 2 years of age. However, the incidence varied widely in relation to age, breed, and genetic constitution and closely paralleled that of toxemia of pregnancy with reference to the same constitutional factors. In addition, all of the tumor bearing animals suffered attacks of this disease during their earlier breeding history. The facts suggest that the association of the two disorders occurred as a result of the liver damage incident to toxemia which impaired the function of this organ in relation to estrin inactivation and the concentration of this substance in the blood stream subsequently rose to a carcinogenic level.

A consideration of the disparity in the distribution of uterine growths in rabbits and women indicated that the absence of squamous epithelium in the cervix of the rabbit and the greater physiological activity of the endometrium of the fundus in this species were the determining factors.

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