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Phenomena of blood coagulation with special reference to a transplanted tumor of the rabbit.

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A certain proportion of rabbits, about 20 per cent, inoculated with the tumor now employed in our work[1] develop a fulminating malignant disease and die with widespread metatases as early as 3 to 5 weeks after inoculation. When these animals are examined at autopsy it is frequently noticed that the blood retained in the heart or in contact with raw surfaces may show little or no tendency to the formation of a firm clot. The contrast between this behavior and the apparently normal formation of blood clots in rabbits of the same group, which develop a more benign form of disease, led to the following quantitative study of the phenomena of blood coagulation in tumor bearing rabbits as a possible factor of importance in determining the course of malignant disease.

It was deemed advisable in beginning this investigation, to approach the problem first from the standpoint of the gross prenomenon of coagulation, separating this into two components, clotting time and clot contractibility. In the measurement of the so-called clotting time, the many methods thus far elaborated take as an endpoint the attainment of a certain consistency of clot in its resistance to one force or another. This necessarily confuses the idea of time *per se* of the initiation of coagulation with that of the firmness or contractibility of the clot and rules out all such methods for the present purpose. It was necessary, therefore, to devise methods and instruments for the investigation of this problem. The details of the methods employed and the description of the instruments used will be reported elsewhere.

For the present, it is sufficient to say that an instrument was devised which made it possible to observe the earliest gross pre-

* Introduced by Wade H. Brown.

[1] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, xxxvii, 601, 631, 799, 811, *ibid.*, xxxviii, 347, 367, 385.

cipitation of fibrin in a thin film of blood. Clotting time determined in this way is expressed in seconds, and the normal for the rabbit lies between 20 and 30 seconds. Under abnormal conditions the time may be diminished to 8 or 10 seconds, or extended as long as 2 or 3 minutes.

For the accurate estimation of clot contractibility it was also necessary to elaborate a procedure, which employs as an index of contractibility the amount of serum expressed from the clot under uniform conditions. The values of serum expressed from the blood clot are translated into terms of percentage of the quantity of plasma present in the whole blood. Measurement of the serum expressed from the clot is made at the end of 1 hour, in order to indicate the rate of contraction of the clot; and again at the end of 6 hours, in order to ascertain the final completeness of contraction. The normal 1 hour expression of serum in the rabbit lies between 82 per cent and 88 per cent of the available plasma, and the final (6 hour) expression between 85 per cent and 90 per cent.

Clotting time and clot contractibility, estimated in this way, have been followed consecutively in normal rabbits, and it has been found that, provided the tests are made in the fasting animal, the results are remarkably constant from day to day. The equilibrium of these phenomena is upset immediately by the advent of any of the spontaneous diseases to which rabbits are prone, such as snuffles, pneumonia, mastoiditis, subcutaneous abscess, and even infestation with vermin.

These gross phenomena of blood coagulation have been investigated systematically in 20 tumor rabbits at intervals of 6 or 8 days from the date of inoculation until the death of the animal or up to the expiration of 2 months. At the same time careful clinical observations were made, including the rate of growth and character of the primary tumor, the occurrence of metastases, and the general physical condition of the animal. At the time of death or at the end of the 2 months period of observation autopsy was performed in detail.

Considered broadly, the clotting time, the rate of clot contraction, and the completeness of clot contraction, behaved in a similar fashion, and for the present purposes the three phenomena are referred to collectively as the efficiency of coagulation. However, there was frequently exhibited a clear-cut

individualism in these values which deserves notice, a tendency displayed by one or another to compensate for failure in the others by assuming an abnormally high value.

It may be stated, in general, that the efficiency of coagulation was found to run parallel with the fluctuations in resistance manifested by the animal against the tumor. With the first local growth and invasion by the primary tumor and at a time when no other evidence of reaction on the part of the host could be recognized, definite alterations were apparent in the blood clotting mechanism. These alterations took various forms, in some of the rabbits there was a sharp rise in the efficiency of clotting time and clot contraction, and in others a fall in the efficiency of these functions. As the disease progressed, a few of the former group maintained the initial hyper-efficiency of coagulation throughout, and it was these animals that recovered completely from the effects of the tumor. On the other hand, a few of the latter group maintained the initial hypo-efficiency of coagulation, and it was these animals, without exception, that died at an early period with the most extreme form of malignancy. The remaining animals of each group did not persist in the initial type of response, but subsequently went through a series of elevations and depressions in clotting efficiency. The depressions that occurred were frequently associated with loss of body weight and strength, and were almost invariably followed in a few days by the appearance of a fresh crop of clinically demonstrable metastases. On the other hand, the occurrence of increased efficiency in coagulation was accompanied by a return of weight and vigor and by a cessation of metastatic deposit. Moreover, those animals which exhibited frequent and extensive depressions in efficiency of coagulation were, in almost every case, ones which developed many metastases and ultimately succumbed to the disease; while those which displayed infrequent and inconsequential depressions usually recovered.

There was one notable exception to this general plan of behavior which deserves emphasis. Occasionally rabbits were found which maintained a fairly high level of clotting efficiency throughout the course of the disease, but, nevertheless, gave way to extensive primary and metastatic tumor involvement. When examined at autopsy, the metastasis was found to be almost entirely of a type suggesting a lymphatic distribution of tumor

cells, in that those organs not directly in the path of the regional lymphatics were untouched. This picture contrasted strikingly with that presented by rabbits, which during the course of the disease displayed the most marked inefficiency of coagulation, for in these the great mass of metastatic deposit was lodged in parts of the body to which it very evidently was carried by the blood stream. On the strength of these extreme examples, a more critical analysis of the other cases brings to light the fact that metastases appearing shortly after a depression in coagulability were chiefly of a blood borne type.

From the work done thus far, it may be concluded that (1) certain characteristics of the blood clotting phenomenon in rabbits, *viz.*, the clotting time and the rate and completeness of clot contractibility, remain remarkably constant in animals free from disease; (2) that alterations in these phenomena occur in rabbits suffering with any acute debilitating disease, including experimental malignancy; and (3) that the alterations in coagulability, as studied in tumor bearing rabbits, tend to run a course parallel with the degree of effort expended by the animal in resisting the inroads of the tumor, more particularly in resisting metastases by way of the blood stream.

In the histological examination of organs, particularly the lungs, of rabbits killed during the progress of malignant disease and in the early metastatic period, it is not uncommon to find tumor-cell emboli lying within the lumina of arterioles surrounded by a thrombus. This has been noted by others in cases of human malignancy.[2, 3] It seems possible that the wall of blood clot, that forms about tumor emboli when they first lodge in a capillary bed, may impair the chances of the tumor cells for taking root in their new position, and, moreover, that the degree of firmness and contraction of which the clot is capable is a factor of considerable importance in determining the survival or death of the cells. It appears likely, therefore, that, not only does the efficiency of coagulation in these rabbits run parallel with the course of events in the disease, but that it also possesses some degree of determining influence.

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[2] Schmidt, M. B., Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome zu den leukämischen Neubildungen, Jena, 1903.

[3] Ewing, J., Neoplastic Diseases, Philadelphia, 1919, Saunders.